INTRODUCTION: Intervertebral disc degeneration is thought to be associated with genetic factors as well as excessive mechanical loading, altering the biomechanical properties of the intervertebral disc. Although the precise molecular biological mechanism of disc degeneration is still unclear, previous studies have suggested that apoptosis of disc cells may play one of the most important roles in disc degeneration. Some studies suggested that disc cells participate in the intrinsic pathway, which subsequently undergo apoptotic cell death through mitochondrial involvement. Among the intrinsic pathway, bcl-2 prevents or delays apoptotic induction by a large variety of stimuli in many cell types. Molecular intervention at the level of bcl-2 in the apoptotic pathway, therefore, has the potential to enhance cell survival. The purposes of this study was to investigate whether bcl-2 overexpression in rat intervertebral disc cells was effective in preventing serum starvation-induced apoptotic cell death and to discuss the potential advantages of this approach to provide a therapeutic benefit in regulating disc degeneration.

METHODS: All animal procedures were performed under the guidance of our animal research committee. Rat nucleus pulposus cells were transfected in vitro with a codon optimized rat bcl-2 gene (pBApo-CMV-bcl-2) or control plasmid vector. Forty-eight hours after transfection, cells were cultured in serum-deprived medium. After serum withdrawal (6 and 48 h), the cells were evaluated for bcl-2 protein levels using Western blotting. Cell apoptosis was also analyzed by a flow cytometer. To investigate the effects of bcl-2 overexpression on the final apoptotic pathways and on basic genes important for nucleus pulposus homeostasis, mRNA levels of caspase-3, type II collagen and aggrecan were also quantified by real-time RT-PCR.

RESULTS: Western blot analyses demonstrated strong induction of Bcl-2 expression in cells transfected with the pBApo-CMV-bcl-2 plasmid, harvested at both 6 and 48 hours after serum starvation. Flow cytometric analyses demonstrated that serum withdrawal significantly increased apoptotic rates in the nucleus pulposus cells compared to untreated control cells in the early stage (6 h) after serum starvation. At 48 h after serum removal, further increases in apoptotic cells were observed in both serum-starved only and negative control plasmid vector groups compared to the untreated control group. Conversely, significant inhibition of apoptosis was seen in bcl-2 transfected group compared to the other serum-starved groups (figure 1).

At 6 h after serum removal, there was a significant increase in caspase-3 mRNA compared to untreated controls. Conversely, at 48 h after serum withdrawal in both serum-starved and control plasmid vector groups, there remained significantly higher caspase-3 mRNA levels compared to untreated control groups. However, caspase-3 mRNA was significantly reduced in cells transfected with bcl-2 compared to other serum-starved groups (figure 2).

mRNA levels of type II collagen and aggrecan were significantly decreased in bcl-2 transfected and control plasmid vector groups compared to untreated controls. However, there remained significantly higher type II collagen and aggrecan mRNA levels in bcl-2 transfected groups compared to control plasmid vector groups (figure 3).

DISCUSSION: There have been no reports regarding transfer of the bcl-2 gene into the intervertebral disc cells. In the current study, rat nucleus pulposus cells were successfully transfected with bcl-2, which effectively reduced apoptotic cell numbers that were induced by serum starvation. These new findings confirm our proof-of-principle to demonstrate that the regulation of apoptotic cell death is possible via exogenous bcl-2 overexpression in nucleus pulposus cells. Also, this study clearly revealed that in vitro overexpression of bcl-2 reduced caspase-3 mRNA levels in rat nucleus pulposus cells. The results of indicated that bcl-2 was also effective in suppressing the later stages of programmed cell death in nucleus pulposus cell.

CONCLUSIONS: The present study was conducted in an attempt to inhibit active cell death to make subsequent gene therapy a more practical concept because disc degeneration is typically accompanied by the loss of disc cells numbers. This new approach to combat degenerative disc disease has not previously been documented and there have been no reports regarding the transfer of the bcl-2 gene into the intervertebral disc cells. We think that this study has important implications in the further development of suitable therapies for degenerative disc disease and provides valuable new information about a novel approach to treat or delay disc degeneration.

REFERENCE: Sudo H et al. J Orthop Res. 2010

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