Transplantation of Activated Nucleus Pulposus Cells after Cryopreservation: Efficacy Study in Canine Disc Degeneration Model

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Disclosures:

Introduction: Tissue disruption and altered function of the intervertebral disc (IVD) leads to progression of degenerative disc disease. Transplantation of viable cells that can function to maintain the production of matrix components of the nucleus pulposus (NP) has been shown to be effective in delaying the course of degeneration. Of the various cell types that has been shown by animal models, NP cells activated by co-culture with direct cell-to-cell contact with bone marrow-derived stromal cells (BMSCs) have found its way to clinical feasibility study 1), 2). The major limitation of this method for application to various spinal pathologies is that cells were activated in 7-day ex vivo culture period and carried out to transplantation at that time. However, if the cells could be cryopreserved after removal, and then thawed and cultured when required by the patient’s condition and extent of disc degeneration, the scope of application would be unconstrained by the short culture time and could be expanded to cover a variety of degenerative diseases that could be treated by transplantation of activated NP cells. In 2013, in vitro analysis reported by Tanaka et al. showed that there were no clear differences between the non-cryopreserved and cryopreserved activated NP cells in terms of cell viability, proliferation capacity, and capacity to synthesize extracellular matrix in vitro 3). However, it is not known whether cryopreserved cells can function effectively in vivo. Therefore, the efficacy of activated nucleus pulposus cell transplantation after cryopreservation in canine disc degeneration model was investigated.

Methods: Animal experiments were carried out under IRB approval. Cartilage dystrophic canine species (10 to 12 month-old beagles, approx. weight 10kg, n = 6) was used. First, lateral radiographs and magnetic resonance imaging (MRI) of lumbar spine were taken, and preexisting vertebral abnormality or disc disease were checked in all animals. Three IVDs (L2/3-L4/5) were assigned in each animal to any of the following three groups (D: degenerated control group, N: non-cryopreserved activated NP cell transplanted group and F: cryopreserved activated NP cell transplanted group). Levels were interchanged between individual animals to minimize level difference effects. L5/6 served as normal control (NC) group with no exposure. Under general anesthesia, first operation was performed through lateral approach to induce disc degeneration and to obtain NP cells from L2/3, 3/4, 4/5 discs. The mean mass of the NP aspirated from each discs was 15.9mg. At the same time, Autologous BMSCs were obtained from the iliac crest. In group F IVDs, NP cells were cryopreserved after enzymatic digestion and BMSCs were cryopreserved. Two-weeks after the first operation, NP cells and BMSCs of group F were thawed, and activated by coculture system with direct cell-to-cell contact with BMSCs. In group N, NP cells were separately harvested from L1/2 disc and subsequently activated with BMSCs. In group D, no cells were transplanted after NP aspiration in the first operation. Three-weeks after the first surgery, cell transplantation was performed at the density of 1×10⁶ cells/100μl in to group N and F discs under fluoroscopic guidance. At 4, 8 and 12 weeks after transplantation, plain radiographs and MRI were taken for evaluation of disc height and signal changes in T2 weighted image. At 12 weeks after transplantation, all beagles were euthanized and discs were harvested for histological analysis. HE and Safranin-O stained sections were studied and extent of disc degeneration was graded based on the system by Nishimura et al 4). All statistical evaluations among four groups were determined using the one way ANOVA and fisher’s PLSD post hoc test. Statistical significance was accepted at P<0.05.

Results: On plain radiograph analysis, DHI decreased rapidly after 3 weeks after surgery in groups D(83.0±3.2%), N(84.8±5.7%) and F(84.5±4.6%) IVDs. The average %DHI of group D was reduced to 69.4±8.9 % at the time of 12 weeks after surgery. In group N IVDs, the average %DHI was reduced to 84.8±5.7%, whereas in group F IVDs similar effect of 83.9±4.2% was confirmed. In both groups N and F IVDs, %DHI were maintained significantly compared to group D IVDs (P<0.01)(Fig.1). Changes in MRI signal intensity using the Pfirrmann classification grading system showed that the grades of groups N and F IVDs were significantly lower than group D IVDs at 12 weeks after transplantation (P<0.01)(Fig.2). In histological analysis group N and F IVDs showed well preserved inner annulus structure, whereas group D IVDs showed revised contour of the inner annulus fibrosus. The average disc grade score using Nishimura and Mochida’s classification system was 3.75 in group D IVDs, while in group N IVDs the score was 1.3 and in group F IVDs it was 2.0 demonstrating that disc degeneration was maintained significantly in both groups N and F IVDs compared to group D IVDs (P<0.01)(Fig.3).

Discussion: The results of the present study demonstrate that transplantation of activated NP cells inhibits progressive disc
Degeneration in canine disc degeneration model. The results of DHI, MRI, and histology showed that there were no significant differences in efficacy between non-cryopreserved versus cryopreserved protocols in vivo. These findings suggest that by using cryopreservation, it may be possible to transplant activated NP cells upon request for patients' needs.

Significance: This is the first report to confirm that transplantation of activated NP cells after cryopreservation can inhibit progression of disc degeneration in vivo. This provides insights into expanding the scope of application of activated NP cell transplantation to cover a range of degenerative disc diseases and to transplant activated NP cells from a cryopreserved cell bank.

Acknowledgments:

Figure 1. Changes in disc height measurement on radiograph

DHI

* P<0.01
Figure 2. Changes in MRI signal intensity
Figure 3. HE and Safranin-O staining at 12 weeks after transplantation

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