STEM CELL THERAPY FOR INTERVERTEBRAL DISC DISEASES IN VITRO AND IN VIVO FEASIBILITY STUDY

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INTRODUCTION Current therapies for degenerative disc disease (DDD) such as surgical discectomy with fusion and disc arthroplasty are aimed at treating the pathologic and disabling conditions arising from DDD (e.g., disc herniation, stenosis, etc.) -- rather than directly treating the underlying problem of disc degeneration. Recent advancements in molecular biology and tissue engineering have made it possible to contemplate directly treating the intervertebral disc itself. Our group and other groups are actively exploring the potential of gene transfer for delaying or reversing the progressive loss of proteoglycans in *early* DDD. Later stages of DDD, however, may require the introduction of new cells to repopulate the disc. Stem cells appear to be excellent candidates for this purpose, based on their ability to differentiate along multiple connective tissue cell lineages. The purpose of this study is to test the feasibility of *stem cell therapy* for the treatment of DDD.

METHODS:

In Vitro Study: Human nucleus pulposus cells (NPCs) were isolated from patients undergoing disc surgery and were co-cultured for 2 weeks with mesenchymal stem cells (hMSCs) from patients undergoing hip surgery using 3-D pellet culture system. Each pellet contained an admixture of 100,000 cells, with NPC-to-MSC ratios of 100:0, 75:25, 50:50, 25:75, 0:100. Proteoglycan synthesis and DNA content were measured by incorporation of ^{35}S and fluourometric analysis, respectively. Histological analysis was also performed using hMSCs retrovirally transduced with Lac Z reporter gene (Over 90% transduction efficiency).

In Vivo Study: Rabbit MSCs (rMSCs) were isolated from bone marrow of a New Zealand White rabbit as previously described[1] and retrovirally transduced with Lac Z reporter gene with five rounds of cell transduction. Nearly 90% of rMSCs were LacZ positive as indicated by X-gal staining. Five rabbits were used for this pilot study. The anterior aspects of the L2-3, L3-4, and L4-5 intervertebral discs were exposed using a retroperitoneal approach under general anesthesia. 100,000 LacZ positive rMSCs suspended in 15 µl saline were injected into L2-3, L3-4 and L4-5 discs. Saline was injected into one rabbit as control disc. Two experimental rabbits were sacrificed at 6 and 12 weeks after transplantation for histological evaluation of the cell viability and potential differentiation.

RESULTS: **In vitro study:** Co-culturing of NPCs with hMSCs in the 3-D pellet culture system resulted in increases in newly synthesized proteoglycans when normalized to DNA content at the ratios of 75:25 and 50:50 as compared with NPCs alone (Figure 1). Histological analysis of the pellets stained with X-gal (counterstained with eosin) showed hMSCs did not undergo extensive proliferation in the pellet. (Figure 2).

In vivo study: A larger number of LacZ positive cells were observed in nucleus pulposus at week 6 and 12 after cell transplantation (Figure 3). The allogenic cells were well tolerated by the recipient rabbits and there was no systemic illness secondary to allogenic transplantation of rMSCs into discs.

DISCUSSION: The data of newly synthesized proteoglycan showed that there was a *synergistic effect* between hMSCs and hNPCs resulting in *upregulated proteoglycan synthesis* in-vitro. The hMSCs co-cultured with hNPCs in the pellet did not appear to undergo proliferation.. The increase in proteoglycan synthesis in the pellets made from the co-culture of the hMSCs and NPCs might be due to the hMSCs differentiating towards NPCs under the influence of some growth factors provided by NPCs or the stimulation of NPCs by agents synthesized by hMSCs, or some other mechanism. Furthermore, in vivo study demonstrated that the transplanted cells were viable within the intervertebral disc up to 12 weeks after transplantation. These encouraging results support the feasibility of developing a *stem cell therapy* approach for the replenishment of cells and creation/maintenance of a more functional extracellular matrix (including proteoglycans) in a degenerated disc. In addition, the in vivo study suggests that mesenchymal stem cells may be potential vehicles for the delivery of therapeutic genes into intervertebral disc. Future studies will determine whether the cells transplanted into the disc differentiate into NPCs and whether they would contribute to the disc repair and regeneration.

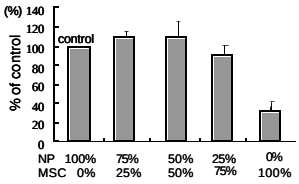

Figure 1. Newly synthesized proteoglycans

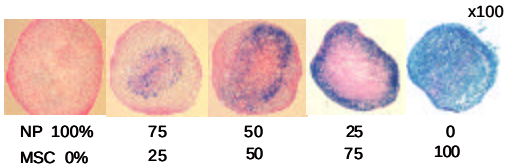

Figure 2. X-gal staining of the pellets containing hNPCs and hMSCs transduced with Lac Z reporter gene

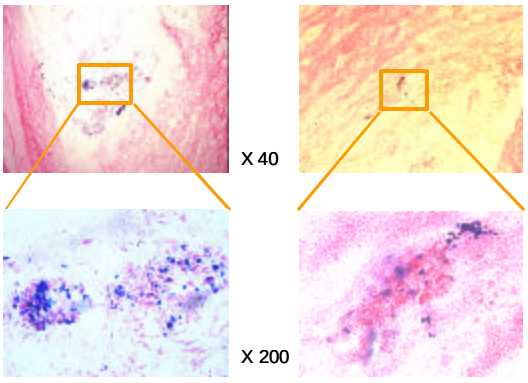

Figure 3. Representative section of intervertebral disc stained with X-gal (counterstained with eosin)
(A) 6 weeks after transplantation of LacZ positive MSCs into intervertebral disc
(B) 12 weeks after transplantation of LacZ positive MSCs into intervertebral disc

Reference:

1. Pittenger MF et al. (1999) Science 284, 143-147