Alleviating disc degeneration progression and spinal ankylosis via allogeneic mesenchymal stem cell transplantation

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INTRODUCTION

Regenerative therapies based on allogeneic stem cells have clinical relevance because of ease of shelf availability and being applicable to individuals whose stem cells might be compromised in terms of regenerative potential. In particular, mesenchymal stem cells (MSCs) are well-suited to allogeneic therapies as they possess potent immunomodulatory activities to escape from allogeneic recognition. Studies of stem cell-based regeneration of the intervertebral disc (IVD) often employ autogeneic cells to avoid immune reactions. Previous allogeneic MSC transplantation studies in normal rat and rabbit IVD reported an apparent lack of immune response after the transplantation. However, the effect of allogeneic transplantation in degenerative models remains unclear. We have previously demonstrated with a mouse tail model that allogeneic stem cell-based IVD regeneration is feasible and the regenerative effects rely on both cell autonomous and non-autonomous mechanisms 1. Nonetheless, tail disc model lacks physiological loading and mechanical interaction with other major musculoskeletal components found in the trunk. Here we used the established puncture-induced rabbit disc degeneration model to test if bone marrow-derived allogeneic MSCs show capacity in arresting disc degeneration in lumbar spine similar to autogeneic MSCs. We aimed to carry out structural and functional characterization as well as long term follow-up to evaluate the safety and efficacy of the transplantation.

METHODS

Progressive IVD degeneration was induced at L2/3 and L4/5 levels by annulus puncture in 6 months old NZW rabbits. Bone marrow MSCs (Ch-MSC) were isolated from Chinchilla Bastard rabbits. The Ch-MSCs were labeled by Qtracker and encapsulated in peptide hydrogel. The composite was directly transplanted into the nucleus pulposus (NP) of the degenerated NZW IVD after 1 month of puncture. PBS or hydrogel alone was injected as controls. Sagittal T2-weighted images were acquired by D2O-assisted 3T MRI for quantification of disc hydration (total DHI) at 1 and 3 months post-treatment (mpt). Lateral spine radiographs were taken to calculate the disc height index (DHI). At 3mpt, the discs were assessed for immune markers and extracellular matrix deposition by immunohistochemistry. Lateral bending, flexion, and extension stiffness, as well as torque of the spinal segments were tested by MTS. Effects of cell dosage and degenerative status on the long term efficacy of Ch-MSCs were studied with hydration and disc height assessments up to 12mpt. At 12mpt the discs were examined by FAST staining. In all analyses, intra-subject L3/4 level was used as normal control and reference.

RESULTS

Ch-MSCs encapsulated in the hydrogel were viable and were able to survive in NP by 3mpt. While the disc height in the control groups gradually decreased (from 100% to 72%DHI), Ch-MSC group showed significant attenuation of disc height loss (to 83%DHI, p<0.05). Ch-MSC group also showed significantly higher hydration content (74%DHI) compared to the controls (54%DHI, p<0.05). Ch-MSC-transplanted animals did not show any adverse morbidity or increased mortality rate. The Ch-MSC-transplanted discs did not show positivity for CD3 and CD56, the markers of lymphocytes and NK cells. Ch-MSC transplantation resulted in increased aggrecan but suppressed collagen I deposition. Transplanted IVD showed increased proteoglycan content and swelling pressure in the NP. While the degenerated segments showed increased bending/rotational stiffness, Ch-MSC-transplantation resulted in significantly lower stiffness in the corresponding motions which were comparable to that of the normal (Fig. 1). Long term follow-up indicated optimal maintenance and recovery of disc hydration at 3mpt of Ch-MSCs (Fig. 2). FAST staining indicated that the Ch-MSC-transplanted discs retained an alcian blue-positive NP resembling that of the normal discs. Transplantation with a high dosage of Ch-MSCs, or transplantation at severe degenerative stage could not arrest the degeneration (Fig. 2).

DISCUSSION

Degenerated discs have modulated inflammatory activity and present hostile micro-environment which potentially compromises the regenerative capacity of MSCs. This study has addressed some vital issues regarding the use of allogeneic MSC for treating disc degeneration and provides insights for future clinical applications. Our findings suggest that allogeneic MSC transplantation can arrest puncture-induced lumbar disc degeneration without evoking host-versus-graft immune response. The transplantation has resulted in a preserved and rehydrated NP compartment. Importantly, a recovery of motion flexibility of spinal segment is observed. Our data imply that the degeneration arrest is associated with preservation of structural and functional integrity in part through inhibition of NP fibrosis and ankylosis.

While MSC transplantation can arrest disc degeneration, progressive disc degeneration resumes in the long term, suggesting that cell-based approaches by themselves may have a limited duration of efficacy. Cell dosage evaluation indicates that an increase in MSC quantity in the transplantation cannot improve the regenerative capacity, but rather negates their effects, implying nutrient supply within the disc may influence the optimal cell dosage for therapy. The lack of discernible effect at severe degenerative stages suggests that the timing of application has a determinative role in the effectiveness of MSCs in alleviating degeneration progression.

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REFERENCES