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Regenerative effects of transplanting mesenchymal stem cells embedded in atelocollagen to the degenerated intervertebral disc

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Abstract

Intervertebral disc (IVD) degeneration, a common cause of low back pain in humans, is a relentlessly progressive phenomenon with no currently available effective treatment. In an attempt to solve this dilemma, we transplanted autologous mesenchymal stem cells (MSCs) from bone marrow into a rabbit model of disc degeneration to determine if stem cells could repair degenerated IVDs. LacZ expressing MSCs were transplanted to rabbit L2–L3, L3–L4 and L4–L5 IVDs 2 weeks after induction of degeneration. Changes in disc height by plain radiograph, T2-weighted signal intensity in magnetic resonance imaging (MRI), histology, immunohistochemistry and matrix associated gene expressions were evaluated between normal controls (NC) without operations, sham operated with only disc degeneration being induced, and MSC-transplanted animals for a 24-week period.

Results showed that after 24 weeks post-MSC transplantation, degenerated discs of MSC-transplanted group animals regained a disc height value of about 91%, MRI signal intensity of about 81%, compared to NC group discs. On the other hand, sham-operated group discs demonstrated the disc height value of about 67% and MRI signal intensity of about 60%. Macroscopic and histological evaluations confirmed relatively preserved nucleus with circular annulus structure in MSC-transplanted discs compared to indistinct structure seen in sham. Restoration of proteoglycan accumulation in MSC-transplanted discs was suggested from immunohistochemistry and gene expression analysis. These data indicate that transplantation of MSCs effectively led to regeneration of IVDs in a rabbit model of disc degeneration as suggested in our previous pilot study. MSCs may serve as a valuable resource in cell transplantation therapy for degenerative disc disease.

Keywords: Intervertebral disc; Nucleus pulposus; Cell therapy; Tissue engineering; Regenerative medicine; Mesenchymal stem cells; Stem cell; Atelocollagen

1. Introduction

Low back pain is a chronic medical problem often associated with work disability and high health care costs [1,2]. Degeneration of the intervertebral disc (IVD), a major cause of low back pain, is an irreversible phenomenon with no currently available treatment [3]. Its etiology is unknown, but it can be described clinically as a loss of proper stability and mobility. Histopathologically, the IVD shows a decrease in water content associated with reduced proteoglycan content of the nucleus pulposus (NP), resulting in destruction of the annular structure and flattening of the disc [4,5]. Disc degeneration occurs naturally with age and circumstances secondary to various spinal disorders [6]. Moreover, there are many surgical procedures, such as

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nucleotomy or a long vertebral fusion, in which degeneration in the operated site or even in the adjacent discs is accelerated, caused by reduction of the nucleus, increased axial loading, insufficient mobility and by other factors. Because almost no regeneration of the NP or the annulus fibrosus (AF) is possible [7], development of global methodologies to treat degenerated discs is needed.

Recent advances in molecular biology have provided new knowledge on the nature of the IVD and disc cells. Experimental studies on disc cell function have enabled scientists and clinicians to develop new approaches for the treatment of disc degeneration and regeneration [8]. Currently, strategies to regenerate the disc focus on restoring the ability to regulate matrix production and to restore the disc tissue. These include strategies involving cytokine and growth factor induction, gene therapy, tissue engineering and cell transplantation therapy [8–16].

Autologous NP cell transplantation has become one of the major techniques in attempts to prevent IVD degeneration in animal models [12–16]. However, in clinical situations, it has been considered difficult for broad application. One reason was that the procedure required more cells than can be harvested from a single disc. To overcome this problem, we have focused on the multi-lineage differentiation potential of mesenchymal stem cells (MSCs) as an alternative cell source for cell transplantation therapy of disc degeneration. There is an increasingly enthusiastic interest in stem cells as potential therapeutic reagents for various degenerative diseases and damaged organs [17]. MSCs are stem cells found in small numbers in the periosteum, cord blood or bone marrow [18]. They possess a unique ability to differentiate into various mesenchymal cell types [19,20]. Experimental MSC transplantation therapies are effective in a variety of diseases including the articular cartilage [20–26], and the induction of articular chondrocytes from MSCs in vitro is well established [27–29]. Because most of the cells that comprise the NP and AF in human adults express a chondrocyte-like phenotype, it is strongly suspected that disc cells are also likely to be induced from MSCs [30–33]. A pilot study on the feasibility of this procedure reported successful cell delivery using an atelocollagen gel as a cell-support scaffold [34]. MSCs were adequately delivered to the NP without major leakage using this approach. The study was followed for up to 8 weeks, and implantation was evaluated by histology and immunohistochemistry. Here, we followed the study up to 24 weeks after transplantation and assessed the regenerative effects of the procedure using plain radiography, magnetic resonance imaging (MRI), macroscopic findings, histology, immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR).

2. Materials and methods

Animal experiments were carried out according to a protocol approved by the Animal Experimentation Committee at our institution. Forty New Zealand white rabbits (mean weight 1.5 kg) were divided into three groups: 10 normal controls (NCs) without operations; 10 sham operated with only disc degeneration being induced; and 20 MSC transplantation animals, which were evaluated at 2, 4, 8, 16 and 24 weeks after transplantation (four at each time point).

2.1. Induction of degeneration in sham and MSC transplantation groups

Degeneration of the disc was induced under inhalation anesthesia using 2.5% isoflurane (Abbott Laboratories, North Chicago, IL, USA) in sham and MSC transplantation group rabbits 2 weeks before cell transplantation. NP tissue (5–8 mg wet weight) was aspirated from the IVDs at regions L2–L3, L3–L4 and L4–L5 using an antero-lateral approach with a 21-gauge needle on a 10 ml syringe, as described previously [34].

2.1.1. MSC isolation and culture

MSCs were isolated from rabbit bone marrow by the gradient isolation of mononuclear cells and cell attachment to tissue culture plastic as described previously [34]. Under inhalation anesthesia, marrow blood was collected by aspiration from the iliac crest of the MSC transplantation group rabbits via an 18-gauge needle, collecting 10 ml of marrow blood into 1000 U of heparin. The marrow blood was filtered through a cell strainer for excluding any fatty tissues and blood clots, and then carefully poured over 20 ml of Nycoprep^{TM} 1.077 Animal (Axis-Shield PoC AS, Oslo, Norway) and centrifuged at 600g for 30 min. Mononucleated cells were recovered from the middle layer, washed three times with phosphate-buffered saline (PBS) and cultured until they reach approximately 80% confluence over about 12–15 days in 25 cm^2 flasks in low-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, Green Island, NY, USA) containing 10% fetal bovine serum (Gibco) and antibiotics (penicillin G, 100 U/ml; streptomycin, 0.1 mg/ml; amphotericin B, 0.25 μg/ml) at 37 °C under 5% CO_2.

2.1.2. In vitro differentiation assays of the MSCs

The MSCs were checked for multi-linage differentiation by adipogenic, chondrogenic and osteogenic differentiation assays as described previously [22]. Briefly, adipogenesis was induced by induction medium including high-glucose DMEM, 100 nM dexamethasone, 200 μM indomethacin (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 10 μg/ml insulin (Sigma Chemical Co., St Louis, MO, USA) and 500 μM 3-isobutyl-1-methylxanthine (Wako). Induction was confirmed by Oil red O staining. For chondrogenesis, MSCs were pelleted by centrifugation (800g, 5 min) and cultured in induction medium including high-glucose DMEM, 1% ITS+ (Beckton Dickinson, Franklin Lakes, NJ, USA), 100 nM dexamethasone, 50 μM ascorbic acid 2-phosphate, 35 μg/ml proline (Sigma) and 5 ng/ml TGF-β1 (Sigma). The pellet was cultured for 21 days, then made into frozen sections, and evaluated by immunostaining for type II collagen and keratan sulfate. Osteogenesis was induced by culturing MSCs monolayered in medium