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Autologous mesenchymal stromal cell transplantation for spinal cord injury: A Phase I pilot study

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Abstract

Background aims. Mesenchymal stromal cell (MSC) transplantation has immerged as promising therapeutic approach to treat spinal cord injury (SCI). In this pilot study, we investigated the safety of intrathecal injection of autologous bone marrow–derived MSCs in nine patients with SCI. *Methods.* Patients with complete SCI at the thoracic level were divided into two groups: chronic (>6 months, group 1) and sub-acute SCI (<6 months, group 2), according to time elapsed since injury. MSCs were isolated by density gradient separation of autologous bone marrow harvested from the iliac crest. Cells were cultured in a Good Manufacturing Practice–compliant facility to produce clinical scale dose. After quality control testing, MSCs were injected back to patients by intrathecal injection. Safety was defined as absence of adverse event and side effects after 1 month after receiving the injection. *Results.* Six patients had chronic SCI with a median duration of 33 months since date of injury (range: 10–55 months), and three patients were in sub-acute phase of disease. Each patient received two or three injections with a median of 1.2×10^6 MSCs/kg body weight. No treatment-related adverse event was observed during median follow-up of 720 days (range: 630–826 days) in group 1 and 366 days (range: 269–367 days) in group 2, respectively. *Discussion.* This pilot study demonstrated that autologous MSCs can be safely administered through intrathecal injection in spinal cord injury patients. Further investigation through randomized, placebo-controlled trials is needed.

Key Words: *clinical trial, mesenchymal stromal cells, spinal cord injury, transplantation*

Introduction

Spinal cord injury (SCI) is a severe neurological disorder that may result in functional deficit and clinical dependency due to paralysis [1]. Despite several advancements, the currently available treatment strategies, such as pharmacological intervention, surgical management and rehabilitation techniques, provide limited results [2]. Cellular transplantation has recently immerged as a possible therapeutic approach to SCI [3]. For this purpose, various cell types have been used in preclinical and limited clinical trials; these include Schwann cells, macrophages, olfactory ensheathing cells and adult stem cells, such as neural stem cells, umbilical cord blood stem cells, mesenchymal stromal cells (MSCs) and induced pluripotent stem cells [4–9].

MSCs are attractive candidates for cellular transplantation strategies. MSCs are found in many adult and fetal tissues. They can be easily obtained from a variety of tissue sources, expanded in long-term cultures without cytogenetic changes, and potentially differentiate into various lineages including neural cells, both *in vitro* and *in vivo* upon induction. MSC transplantation in experimental models of SCI resulted in functional recovery by increasing area of tissue sparing, remyelination of spared white matter and enhanced axonal regeneration [10]. According to recent work, MSCs accomplish therapeutic effect primarily by secretion of neurotrophic factors and provision of stromal support to augment repair at site of spinal injury [11]. We present here a pilot study on the safety of autologous transplantation of *ex vivo* expanded autologous BM-MSCs in a Pakistani cohort of spinal cord injury patients.

Methods

Selection of patients

This pilot study was designed to determine the safety and feasibility of autologous transplantation of MSCs in spinal cord injury patients. The study was approved

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by institutional review boards and ethical committees of both participating hospitals under guidelines of Pakistan Medical Research Council (PMRC) and registered at clinicaltrials.gov (NCT02482194). Nine patients suffering from sub-acute or chronic traumatic spinal cord injury of thoracic level were included in the study. All patients had complete spinal cord transection resulting in ASIA scale A impairment. The patients were divided into two groups: chronic (>6 months, group 1) and sub-acute SCI (<6 months, group 2), according to time elapsed since injury. An initial stabilization and decompression was carried out by surgery. After healing of wounds, these patients were offered option of autologous stem cell transplantation, and informed written consent was obtained from each patient in compliance with Helsinki declaration.

Expansion of bone marrow MSCs using pooled human platelet lysate

A volume of 50–60 mL bone marrow aspirate was collected from patients' iliac crest under aseptic conditions. The aspirate was diluted 1:1 in phosphate buffered saline (PBS; Gibco), layered slowly over lymphocyte separation medium (LSM; 1.077 g/L; MP Biomedicals) and centrifuged at 450*g* for 30 min. Mononuclear cells (MNCs) were collected from plasma-Ficoll interface and seeded at a density of 10^5 cells/cm^2 in 175-cm^2 culture flasks (Corning) containing growth medium and incubated at 37°C in a humidified environment with 5% CO_2. The growth medium comprised Dulbecco's Modified Eagle's Medium (DMEM) with low glucose and Glutamax (Gibco) supplemented with 10% pooled human platelet lysate and 2 U/mL heparin, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 0.25 ug/mL amphotericin-B (Sigma Aldrich). The batches of pooled platelet lysate were prepared as described previously [12]. After 48 hours of incubation the non-adherent cells were removed by changing medium. Medium was then changed twice weekly. MSCs appeared as plastic-adherent, spindle-shaped cells in 8–10 days. Cells were harvested upon reaching 60–80% confluence, using 0.25% TryPLE Select solution (Gibco) and passaged at density of 1000 cells/cm^2. Clinically relevant dose was achieved in 4 weeks of culture and reseeding, after which cells from second passage (P_2) cells were harvested.

Colony-forming unit assay

Colony-forming unit (CFU) fibroblast assays were carried out by seeding bone marrow MNCs at low density (10^5 cells/cm^2) in T25 flasks and cultured for 14 days at 37°C and 5% CO_2, changing the medium twice weekly. After 14 days of culture, medium was removed, and cells were fixed and stained with 1% crystal violet in 100% methanol. Colonies containing ≥50 cells were counted and calculated as fraction of per million MNCs seeded.

Immunophenotyping of cultured MSCs

MSCs harvested from second passage were washed twice with PBS and adjusted to 1×10^6 cells/mL in fluorescence-activated cell sorting (FACS) buffer (PBS with 1% FBS and 1% sodium azide). To each tube, 100 µL of cell suspension was added and incubated for 20 min at 4°C with anti-human-antigen monoclonal antibodies or with their appropriate isotype-matched controls. CD34-PE (clone 581), CD45-FITC (clone H-130), CD73-PE (clone AD2), CD90-FITC (clone 5E10), and CD105-PerCP Cy5.5 (clone 266) were used for immunophenotyping of MSCs (all clones from BD Biosciences), whereas 7-amino-actinomycin D (7AAD) staining was performed to determine the percentage of viable cells. Fluorochrome-labeled mouse isotype controls were used to adjust instrument settings.

After incubation, cells were washed twice with PBS and resuspended in FACS stain buffer. Cell acquisition was carried out on BD FACS Calibur cytometer and analyzed on FACS CellQuest software (BD Biosciences).

Trilineage differentiation of MSCs

Osteogenic differentiation

Induction of MSCs into osteogenic lineage was achieved by using StemPro osteogenesis differentiation kit from GIBCO (Life Technologies). MSCs were cultured in induction medium for 21 days with medium change twice weekly. After 21 days, cells were washed with PBS, fixed with 4% formaldehyde solution and stained with 2% alizarin red to assess calcium deposition in osteocytes.

Chondrogenic differentiation

For chondrogenic induction, micro-mass culture was generated by seeding 5 µL of cell suspension in a multiwell plate. The culture was incubated for 2 hours in high humidity, after which pre-warmed StemPro chondrogenesis differentiating medium was added to the culture vessel and incubated for 37°C at 5% CO_2 for at least 2 weeks with the medium change every third day. After 14 days, the chondrogenic pellet was processed for alcian blue staining.

Adipogenic differentiation

MSCs from early passage were seeded 1×10^4/cm^2 in a 12-well plate and fed with growth medium for 3–4 days or until they became confluent. After reaching confluence, medium was replaced with StemPro adipogenesis differentiating medium and incubated for