Neuroscience Letters 668 (2018) 138–146

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Concomitant use of mesenchymal stem cells and neural stem cells for treatment of spinal cord injury: A combo cell therapy approach

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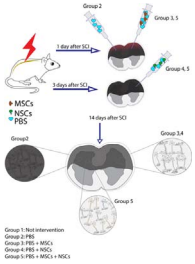
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Cell therapy
Mesenchymal stem cell
Neural stem cell
Spinal cord injury
Stem cell
Transplantation

ABSTRACT

Treatment: with neural stem cells (NSCs) provides a hope to recover the neural damage and compensate for the lost neural structures for restoration of interrupted neural communications above and below the site of injury. However, cell-based therapy approach suffers from many biological barriers and technical caveats which severely hamper the prognosis. The biochemically-rich microenvironment at the site of spinal cord injury (SCI), the continuing neuro-degenerative process and infiltrating immune cells offer a serious barrier to the donor cells. We hypothesized that mesenchymal stem cells (MSCs) concomitantly delivered with NSCs would significantly enhance the effectiveness of cell-based therapy for SCI. In a rodent model of SCI (n = 15 animals/group), MSCs labeled with PKH67 (green fluorescence dye) were delivered on day1 after SCI whereas the same animals were treated with NSCs during the subacute phase on day3 (group-5). In comparison with untreated control (group-1), sham group (without cell treatment; group-2), MSCs alone (group-3) and NSCs alone treated animals (group-4), the combined cell treated animals (group-5) showed significantly higher homing of cells at the site of injury during *in vivo* imaging. Caspase-3 activity was lower in group-5 ($P < 0.05$ *vs* all groups) with concomitant reduction in the pro-inflammatory cytokines IL-1β and IL-6 ($P < 0.05$ *vs* all groups). All cell therapy groups showed significant improvement in neurological function as compared to group-2, however, it was highest in

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https://doi.org/10.1016/j.neulet.2018.01.008
Received 26 September 2017; Received in revised form 3 January 2018; Accepted 4 January 2018
Available online 06 January 2018
0304-3940/

group-5 ($P < 0.05$ *vs* all groups). In conclusion, combined treatment with (NSCs + MSCs) enhances NSCs survival and functional recovery in SCI and is superior to the treatment with either of NSCs or MSCs alone.

1. Introduction

Spinal cord injury (SCI) interrupts physiological communication between brain and the body leading to pathological conditions encompassing both motor and sensory impairment due to neuronal damage. According to the 2016 reports released from National Spinal Cord Injury Statistical Centre, USA (NSCISC) and WHO, there are 17,000 new cases of SCI every year with a total of 282,000 alive patients only in the USA and 500,000 new cases are added every year around the world thus incurring a massive management and financial burden on the healthcare system [1,2].

Repairing spinal cord injury (SCI) remains a therapeutic challenge due to the limited availability of treatment methods other than contemporary approach of symptomatic treatment that may include pharmacological, invasive surgical and rehabilitation approaches. These current therapeutic options for acute SCI are merely supportive and are limited to prevent the progression of the injury and alleviation of its relevant symptoms [3]. With recent advances in regenerative medicine, cell-based therapy has emerged as a novel treatment opportunity for effective treatment of SCI [4]. Extensive studies in the preclinical experimental animal models of SCI and clinical studies in human patients have shown encouraging results from cell-based therapy to treat SCI [5,6]. Despite immense progress, choice and sourcing cells for transplantation remains an area of interest and for that matter, cells from various sources have been used including neural stem cells (NSCs), bone marrow (BM) derived mesenchymal stem cells (MSCs), embryonic stem cells (ESCs) and their derivative cells [7–10]. More recently, induced pluripotent stem cells (iPSCs) and their derivative neuroepithelial-like cells and neural precursor cells have been successfully used in experimental animal models [11,12]. However, the option of using iPSCs and their derivative cells is not with serious issues pertaining to genetic and epigenetic abnormalities and tumorigenic differentiation. Given their inherent tri-lineage differentiation potential to adopt morpho-functional phenotypes of neurons, oligodendrocytes and astrocytes, NSCs seems an obvious choice that could regenerate central nervous system cells [13]. Data emanating from experimental animal studies have shown that NSCs isolated from the sub-ventricular zone, embryonic ganglion eminence, *etc*. effectively repair central nervous system damages [7]. Efforts are underway to further enhance the reparability of NSCs through various means including genetic modification, and combining NSCs with other cell types [7,14–16]. More recently, we have reported that NSCs combined with BM derived MSCs for concomitant delivery of the two cell types was more effective in the treatment of brain stroke in experimental rodent model as compared to treatment with either of the cell alone [17,18]. The features that make MSCs as appropriate source for cell therapy for SCI in combination with NSCs include their ease of availability and culture, low immunogenicity, immunomodulation, trans-differentiation to myelin forming cells and paracrine secretion of neurotrophic factors, *i.e.*, BDNF, bFGF, *etc*. [19,20]. The present study was designed to combine the anti-inflammatory effects of MSCs in early phase (one day after spinal cord injury) and the ability of NSCs to differentiate to neural lineages 3 days after SCI. We hypothesized that the serial combined effects of the two cell types would be more effective than treatment with either of the cell type alone.

2. Materials and methods

The present study conformed to the Guideline for the Care and Use of Laboratory Animals and all the experimental animal procedures were performed strictly in accordance with protocol approved by Ethical Committee of Shiraz University of Medical Sciences, Iran. All surgical manipulations were carried out under general anesthesia. The results shown in the manuscript are replicate of three experiments. All experimental animals had free access to food and water during the course of the experiments.

2.1. Experimental design

Seventy-five Sprague-Dawley male rats weighing 250–300 g were selected and divided randomly into five groups (n = 15) for their respective treatment as detailed below:

Group-1 = Control group, received no treatment and intervention. Group-2 = Sham operated group which underwent spinal cord injury surgery however, basal medium without any stem cell treatment. Group-3 = MSCs group which systemically received BM derived MSCs on day1 after SCI. Group-4 = NSCs group which intra-lesion received NSCs on day3 after SCI. Group-5 = Combined treatment group which received MSCs systemically on day1 after SCI and NSCs intra-lesion on day3 after injury.

2.2. Isolation and expansion of MSCs

BM derived MSCs were isolated from young (8-week old) male Sprague-Dawley rats as described earlier [21]. Briefly, the femur and tibia were removed and their marrow was obtained by flashing them with complete MSCs culture media (DMEM/F12 supplemented with 10% fetal bovine serum (FBS) and 1% pen/strep). The cell suspension was transferred in T-25 cell culture flask and kept at 37 °C and 5% CO_2. The culture media was changed after 48 h and the plastic adherent cells were cultured to confluence and passaged regularly. The cells were characterized for surface markers expression and osteogenic and adipogenic differentiation using the Miltenyi Biotech differentiation media (Cat# 130-091-677 and 130-091-678).

2.3. Isolation and characterization of NSCs

The rat NSCs were isolated form 14-day old embryo. Briefly, the ganglion eminence of the embryo was dissected and the tissue was dissolved in NSCs culture media DMEM/F12 (Invitrogen Cat#10565018), 2% B27 (Gibco Cat#17504044), 1% N2 (Invitrogen Cat#17502048), 10 ng/ml bFGF (Sigma Cat#F0291), 20 ng/ml EGF (Sigma E9644). After 7 days in culture, neurospheres appeared in the culture. The cells were characterized by induction to differentiate by adding 2% fetal bovine serum and immunocytochemically identified using anti-MAP-2 specific antibody (Abcam; Cat#ab5392) for detection of neurons, anti-GFAP specific antibody (Dako Cytomation Cat #Z0334) for detection of astrocytes and anti-CNPase (Abcam Cat# ab6319) for detection of oligodendrocyte. The primary antigen-antibody reaction was detected using specific fluorescently labeled secondary antibodies. The nuclei were visualized by DAPI staining (Millipore, Billerica, MA, USA; Cat# 124653; 1:1000) and observed under fluorescence microscope (Olympus BX53; Shinjuku, Tokyo, Japan) for imaging.

2.4. Experimental model of SCI, cell delivery and in vivo tracking

The rodent experimental model of SCI was developed using our established protocol in the lab as described earlier [7,22]. Briefly, the rats were anesthetized generally with halothane in mixture of N_2O and