The Spine Journal 16 (2016) 764–769

Basic Science

Local versus distal transplantation of human neural stem cells following chronic spinal cord injury

Ivan Cheng, MD[a,*], Michael Githens, MD[a], Robert L. Smith, PhD[a], Tyler R. Johnston, MD[a], Don Y. Park, MD[b], Michael P. Stauff, MD[c], Nima Salari, MD[a], Kali R. Tileston, MD[a], Alexander I. Kharazi, PhD[d]

[a]*Department of Orthopaedic Surgery, Stanford University School of Medicine, 450 Broadway St, MC 6342, Redwood City, CA 94063, USA*
[b]*Department of Orthopaedic Surgery, University of California Los Angeles, 1250 16th St, Santa Monica, CA 90404, USA*
[c]*Department of Orthopaedics and Physical Rehabilitation, University of Massachusetts Medical School, 119 Belmont St, Worcester, MA 01605, USA*
[d]*Stemedica, 5375 Mira Sorrento Place, Suite 100, San Diego, CA 92121, USA*

Received 8 July 2015; revised 10 November 2015; accepted 2 December 2015

Abstract

BACKGROUND CONTEXT: Previous studies have demonstrated functional recovery of rats with spinal cord contusions after transplantation of neural stem cells adjacent to the site of acute injury.
PURPOSE: The purpose of the study was to determine if the local or distal injection of neural stem cells can cause functional difference in recovery after chronic spinal cord injury.
STUDY DESIGN/SETTING: Twenty-four adult female Long-Evans hooded rats were randomized into four groups, with six animals in each group: two experimental and two control groups. Functional assessment was measured after injury and then weekly for 6 weeks using the Basso, Beattie, and Bresnahan locomotor rating score. Data were analyzed using two-sample *t* test and linear mixed-effects model analysis.
METHODS: Posterior exposure and laminectomy at the T10 level was used. Moderate spinal cord contusion was induced by the Multicenter Animal Spinal Cord Injury Study Impactor with 10-g weight dropped from a height of 25 mm. Experimental subjects received either a subdural injection of human neural stem cells (hNSCs) locally at the injury site or intrathecal injection of hNSCs through a separate distal laminotomy 4 weeks after injury. Controls received control media injection either locally or distally.
RESULTS: A statistically significant functional improvement in subjects that received hNSCs injected distally to the site of injury was observed when compared with the control (p=.042). The difference between subjects that received hNSCs locally and the control did not reach statistical significance (p=.085).
CONCLUSIONS: The transplantation of hNSCs into the contused spinal cord of a rat led to significant functional recovery of the spinal cord when injected distally but not locally to the site of chronic spinal cord injury.

Keywords: Chronic; Chronic spinal cord injury; Functional analysis; Neural stem cells; Stem cell transplantation; Spinal cord injury

FDA device/drug status: Not approved for this indication (Neural stem cells).

Author disclosures: ***IC:*** Royalties: NuVasive (F), outside the submitted work; Private Investments: Cytonics (D), Spine Innovations (D), outside the submitted work; Consulting: NuVasive (D), Globus Medical (C), Stryker Spine (B), outside the submitted work; Deputy Editor: *The Spine Journal*. ***MG:*** Nothing to disclose. ***RLS:*** Nothing to disclose. ***TRJ:*** Nothing to disclose. ***DYP:*** Nothing to disclose. ***MPS:*** Research Support (Investigator Salary, Staff/Materials): Pfizer (E, Paid to the institution), outside the submitted work; Deputy Editor: *The Spine Journal*. ***NS:*** Nothing to disclose. ***KRT:*** Nothing to disclose. ***AIK:*** Stock Ownership: Stemedica Cell Technologies (G), outside the submitted work; Employment: Stemedica Cell Technologies (F).

The disclosure key can be found on the Table of Contents and at www.TheSpineJournalOnline.com.

* Corresponding author. Department of Orthopaedic Surgery, Stanford University School of Medicine, 450 Broadway St, MC 6342, Redwood City, CA 94063, USA. Tel.: +1-650-721-7619; fax: +1-650-721-3470.
E-mail address: ivan.cheng@stanford.edu (I. Cheng)

http://dx.doi.org/10.1016/j.spinee.2015.12.007
1529-9430/

Introduction

Spinal cord injury (SCI) is a devastating clinical problem with significant neurologic consequences leading to long-lasting functional disability and paralysis. Limited treatment options exist and most are ineffective in restoring neurologic function. The injured adult mammalian central nervous system normally does not support regrowth of damaged axons. Interruption of myelinated tracts of the central nervous system typically results in irreversible functional deficits. Stem cell transplantation is a promising technology that targets the fundamental pathologic process of axonal degeneration, neuronal loss, and demyelination in SCI [1]. The goal of stem cell transplantation is to replace lost neurons, reconnect interrupted axonal connections, and provide neuroprotective factors to allow for healing and recovery after SCI.

Neural stem cells (NSCs) are a promising source for cell therapies, primarily due to their self-renewal and pluripotentiality [1]. Various studies have shown improved function in the acute SCI model using stem cell therapy. Schira et al. [2] grafted unrestricted somatic stem cells into the vicinity of a dorsal hemisection injury at the thoracic level, and resulted in reduction in lesion size and augmented tissue sparing, enhanced axon regrowth, and significant functional locomotor improvement. Whereas there are other studies demonstrating similar improvement in function after SCI, none have examined both a less-invasive route of cell administration and the timing of cellular therapy [3–6]. These are potentially significant factors in clinical scenarios when patients may receive delayed intervention or may not be able to undergo acute surgical treatment.

It has been suggested that delayed treatment results in more permissive conditions for survival of transplanted cells and for spinal cord regeneration, providing a more hospitable environment for the transplanted cells, and this may be because of decreased inflammation and inflammatory mediators in the subacute phase after SCI compared with the acute stage [7,8]. The chronic phase of SCI has been elucidated by histologic studies analyzing glial scar formation and neuroregenerative potential, and has been determined to be >28 days in the rat model [9]. Parr et al. [10] were able to demonstrate increased survival of directly transplanted stem cells in the subacute phase compared with acute or chronic administration of cells, but functional data were available only for the acute group.

Our laboratory has previously been able to determine that the acute transplantation of human neural stem cells (hNSCs) into the contused spinal cord of a rat leads to significant functional recovery of the spinal cord, when injected either locally or distally to the site of SCI [11]. As of yet, no studies have been performed comparing not only chronic transplantation of neuronal stem cells but also the intrathecal route of administration in a rat contusion SCI model, with analysis of functional scores.

Methods

Before inception of the experiment, approval was obtained from the Institutional Review Board and Administrative Panel on Laboratory Animal Care of Stanford University. Subjects comprised 77-day-old adult female Long-Evans hooded rats (200–350 g; Charles River Laboratories, Wilmington, MA, USA). Four groups in total were identified: two experimental and two control groups. A power analysis was conducted, and it was determined that a minimum of six subjects in each group was required to detect a 3-point difference on the Basso, Beattie, and Bresnahan (BBB) locomotor scoring system [12]. All subjects sustained a moderate contusion SCI.

Human neural stem cells were collected from a single donor as previously described [11]. The cells (10^5 cells/mL) were seeded in serum-free medium with neural basal medium and supplements. The components included the following: Neurobasal (96%; Gibco/Invitrogen, Grand Island, NY, USA), GlutaMAX (1%; Gibco/Invitrogen), heparin (8 mg/mL; Sigma-Aldrich, St. Louis, MO, USA), basic fibroblast growth factor and epidermal growth factor (bFGF: 20 ng/mL; EGF: 20 ng/mL; human, recombinant; Chemicon International, Temecula, CA, USA), and leukemia inhibitory factor (10 ng/mL; human, recombinant; Chemicon International). For routine passaging, TrypLE (Gibco/Invitrogen) was used as the dissociating agent.

For viral transfection, hNSCs were passaged and the neurospheres were dissociated. Of the cell culture supernatant, 5 mL was collected and saved for proteomics analysis. Cell culture supernatant was collected with Hank balanced salt solution (Invitrogen, Carlsbad, CA, USA) rinses and centrifuged at 200×*g* for 5 minutes at room temperature with 50% brake to collect non-adherent cells. Of warmed TrypLE (Invitrogen), 10 mL was added to each flask and returned to the incubator for 10 minutes. Ten milliliters of warmed neural growth medium (NGM [Invitrogen] with 1% 100x GlutaMAX-100 [Invitrogen]), 10 μg human basic fibroblastic growth factor, 10 μg human epidermal growth factor, 0.6% heparin sodium injection 1000 USP/mL (APP Pharmaceuticals, Schaumburg, IL, USA), 0.1% Fetal Clone III (Invitrogen), and 2% 50x B27 supplement without Vitamin A (Invitrogen) were then added, and the cells were removed and homogenized by pipetting to dissociate the neurospheres. The hNSCs were washed in Hank balanced salt solution and centrifuged at 300×g for 5 min at room temperature with 50% brake. The hNSCs was resuspended in a small volume of NGM and added to a 24-well plate for transfection. Lentiviral vector with the CMV promoter or luciferase reporter gene was added to the wells at the desired multiplicity of infection of 50. The hNSCs were incubated with the vector particles for 24 to 48 hours or until the desired fluorescence was obtained. Fresh media was added, and 24 hours later the cells were analyzed using bioluminescence. Two days before transplantation, neurospheres (Fig. 1) were enzymatically dissociated into single cell suspensions and cultured in fresh medium. Experimental groups received a minimum of 5×10^5