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Basic Science

Spinal motor neurite outgrowth over glial scar inhibitors is enhanced by coculture with bone marrow stromal cells

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Abstract

BACKGROUND CONTEXT: Transplantation of bone marrow cells into spinal cord lesions promotes functional recovery in animal models, and recent clinical trials suggest possible recovery also in humans. The mechanisms responsible for these improvements are still unclear.

PURPOSE: To characterize spinal cord motor neurite interactions with human bone marrow stromal cells (MSCs) in an in vitro model of spinal cord injury (SCI).

STUDY DESIGN/SETTING: Previously, we have reported that human MSCs promote the growth of extending sensory neurites from dorsal root ganglia (DRG), in the presence of some of the molecules present in the glial scar, which are attributed with inhibiting axonal regeneration after SCI. We have adapted and optimized this system replacing the DRG with a spinal cord culture to produce a central nervous system (CNS) model, which is more relevant to the SCI situation.

METHODS: We have developed and characterized a novel spinal cord culture system. Human MSCs were cocultured with spinal motor neurites in substrate choice assays containing glial scar–associated inhibitors of nerve growth. In separate experiments, MSC-conditioned media were analyzed and added to spinal motor neurites in substrate choice assays.

RESULTS: As has been reported previously with DRG, substrate-bound neurocan and Nogo-A repelled spinal neuronal adhesion and neurite outgrowth, but these inhibitory effects were abrogated in MSC/spinal cord cocultures. However, unlike DRG, spinal neuronal bodies and neurites showed no inhibition to substrates of myelin-associated glycoprotein. In addition, the MSC secretome contained numerous neurotrophic factors that stimulated spinal neurite outgrowth, but these were not sufficient stimuli to promote spinal neurite extension over inhibitory concentrations of neurocan or Nogo-A.

CONCLUSIONS: These findings provide novel insight into how MSC transplantation may promote regeneration and functional recovery in animal models of SCI and in the clinic, especially in the chronic situation in which glial scars (and associated neural inhibitors) are well established. In addition, we have confirmed that this CNS model predominantly comprises motor neurons via immunocytochemical characterization. We hope that this model may be used in future research to test various other potential interventions for spinal injury or disease states.

Keywords: Bone marrow stromal cells; Spinal motor neurites; Glial scar nerve inhibitors; Spinal cord injury; In vitro modelling; Secretomes

FDA device/drug status: Not applicable.

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Introduction

Injury to the central nervous system (CNS) usually initiates a poor intrinsic regenerative response for a number of reasons. Immune reactions, which in other tissues may help to recruit reparative cells, often have a devastating effect on CNS tissue function. Inflammation and ensuing secondary cascades can cause extensive neuronal and glial cell death, as well as glial cell activation and hypertrophy [1]. In an effort to restore the blood-brain barrier, astrocytes at the site of injury become reactive and synthesize a proteoglycan-rich matrix [2]. Myelin debris–associated molecules, including Nogo-A and myelin-associated glycoprotein (MAG), are also released from damaged neural tissues [3]. These events combine to produce a hostile environment for nerve regrowth [2–6].

There has been extensive interest worldwide in the development of cell transplantation strategies for the treatment of CNS damage, in particular spinal cord injury (SCI). Many diverse potential cell therapies have been tested, each targeting different distinct stages of SCI and mechanisms of spinal cord repair [7–10]. Allogeneic embryonic stem cells and umbilical cord–derived cells, as well as possible autologous cell sources, including adult neural stem cells, Schwann cells, and olfactory ensheathing cells, have been shown to promote axonal regeneration and restore function in animal models of SCI [11–17]. These types of cell are thought to act in a number of ways depending on the cell type transplanted, including replacing dead or damaged neurons and glia, reestablishing neural networks, remyelinating demyelinated axons, and reducing the hostile nature of the SCI lesion.

Autologous cell therapies derived from bone marrow have also been shown to enhance functional recovery in animal models of SCI and possibly in the clinic [10], but the repair mechanisms responsible are still largely unclear. Some controversial evidence exists which suggests that bone marrow cells, including marrow stromal cell (MSC) and hematopoietic stem cell fractions, may transdifferentiate to replace lost neurons and glia, in a manner similar to that proposed for embryonic stem cells and neural stem cells [18–22]. However, the consensus of opinion seems to be that for MSC transplantation at least, the most likely mode of activity is an induction of a diverse myriad of paracrine anti-inflammatory pathways and directly restorative cell-matrix and cell-cell interactions [23–29].

Previously, we have used growth substrate choice assays to examine how human MSCs influence neurite outgrowths from explants of chick dorsal root ganglia (DRG). We have demonstrated that MSCs help neurites to overcome the effects of some of the major nerve-inhibitory molecules found in SCI lesions, including neural proteoglycans, Nogo-A, and MAG [30]. This established model of sensory nerve growth provided an excellent platform to examine in real-time possible cell-matrix and cell-cell interactions that may occur in the SCI milieu. In the present study, we have adapted and refined our system by replacing DRG explants with spinal cord cultures to provide a more relevant model of CNS nerve growth. We envisage that the establishment of a novel spinal nerve growth substrate assay, which comprises characterized motor neurons and relevant neural matrix molecules, will provide an invaluable research tool for testing SCI therapeutics, which will have further applications in the broader fields of CNS tissue engineering and repair.

Materials and methods

Ethics statement

All research involving human participants was completed with written informed consent and local research ethics committee approval: Shropshire & Staffordshire Strategic Health Authority (reference number: 04/02/RJH). Ethical approval and a Home Office project license for the study were not required under the United Kingdom Animal (Scientific Procedures) Act of 1986 because chicks were killed by decapitation (which is an appropriate method under Schedule 1 of the Act).

Human bone MSC culture

Bone marrow aspirates or bone chips were harvested from the iliac crest of individuals undergoing spinal fusion in the treatment for lumbar degenerative disorders (n=5; aged 29–53 years). Bone marrow aspirates and bone chips were kindly collected by spinal surgeons from the Centre for Spinal Disorders and sent to the spinal studies research laboratories for processing (both based at the Robert Jones and Agnes Hunt Orthopaedic Hospital Orthopaedic Hospital, Oswestry, UK).

Bone chips were perfused with Dulbecco Modified Eagle's Medium (DMEM/F12) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin (P/S) (Invitrogen Life Technologies, Paisley, UK). Mononuclear cells isolated by density gradient centrifugation at 900 *g* for 20 minutes over Lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway) were plated out in DMEM/20% FBS+P/S medium (Invitrogen Life Technologies) at a seeding density of 20×10^6 cells per flask. After 24 hours, nonadherent cells were removed, and the adherent cell populations were cultured in monolayer and were maintained in a humidified atmosphere of 5% CO_2 at 37°C through to Passages II and III in DMEM/10% FBS+P/S medium. Marrow stromal cell cultures used in this study were characterized according to the MSC CD immunoprofile criteria published by the International Society for Cellular Therapy [31].

Embryonic chick neuronal cultures

Spinal cords were dissected from Day 4.5 hybrid brown chicks as described previously [32] and cut into 10 to 20