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A comparison of the behavioral and anatomical outcomes in sub-acute and chronic spinal cord injury models following treatment with human mesenchymal precursor cell transplantation and recombinant decorin

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ABSTRACT

This study assessed the potential of highly purified (Stro-1$^+$) human mesenchymal precursor cells (hMPCs) in combination with the anti-scarring protein decorin to repair the injured spinal cord (SC). Donor hMPCs isolated from spinal cord injury (SCI) patients were transplanted into athymic rats as a suspension graft, alone or after previous treatment with, core (decorin^{core}) and proteoglycan (decorin^{pro}) isoforms of purified human recombinant decorin. Decorin was delivered via mini-osmotic pumps for 14 days following sub-acute (7 day) or chronic (1 month) SCI. hMPCs were delivered to the spinal cord at 3 weeks or 6 weeks after the initial injury at T9 level. Behavioral and anatomical analysis in this study showed statistically significant improvement in functional recovery, tissue sparing and cyst volume reduction following hMPC therapy. The combination of decorin infusion followed by hMPC therapy did not improve these measured outcomes over the use of cell therapy alone, in either sub-acute or chronic SCI regimes. However, decorin infusion did improve tissue sparing, reduce spinal tissue cavitation and increase transplanted cell survivability as compared to controls. Immunohistochemical analysis of spinal cord sections revealed differences in glial, neuronal and extracellular matrix molecule expression within each experimental group. hMPC transplanted spinal cords showed the increased presence of serotonergic (5-HT) and sensory (CGRP) axonal growth within and surrounding transplanted hMPCs for up to 2 months; however, no evidence of hMPC transdifferentiation into neuronal or glial phenotypes. The number of hMPCs was dramatically reduced overall, and no transplanted cells were detected at 8 weeks post-injection using lentiviral GFP labeling and human nuclear antigen antibody labeling. The presence of recombinant decorin in the cell transplantation regimes delayed in part the loss of donor cells, with small numbers remaining at 2 months after transplantation. In vitro co-culture experiments with embryonic dorsal root ganglion explants revealed the growth promoting properties of hMPCs. Decorin did not increase axonal outgrowth from that achieved by hMPCs.
We provide evidence for the first time that (Stro-1$^+$) hMPCs provide: i) an advantageous source of allografts for stem cell transplantation for sub-acute and chronic spinal cord therapy, and (ii) a positive host microenvironment that promotes tissue sparing/repair that subsequently improves behavioral outcomes after SCI. This was not measurably improved by recombinant decorin treatment, but does provide important information for the future development and potential use of decorin in contusive SCI therapy.

Introduction

Following (SCI) there is minimal regenerative capacity to restore effective functional connectivity of disrupted descending motor and ascending sensory pathways. Cell death and secondary injury cascades, as well as an overall negative balance between factors that inhibit or promote axon growth and remyelination in the adult CNS remain some of the major hurdles associated with spinal cord repair. Multipotent precursor cells (MPCs) or mesenchymal cells (MSCs) routinely taken from the stromal tissue of bone marrow (Gronthos and Simmons, 1996) have gained widespread support in therapeutic application in SCI for the promotion of tissue sparing, axon regeneration and functional recovery (Hodgetts et al., 2013; Nandoe et al., 2006). However, there is continuing speculation as to the contribution of transplanted MSCs to SC regenerative events, especially with

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low levels of cell engraftment and a lack of convincing evidence for transdifferentiation into neuronal phenotypes in vivo (Phinney and Prockop, 2007). MSCs have the advantage of relative ease of isolation from bone marrow aspirates, in vitro cultivation, multipotent differentiation potential, immunosuppressive properties and their applicability in both the autologous and allogeneic transplant settings. It is difficult to compare and contrast existing studies using MSCs, as some of the populations of rat or mouse cells used were heterogeneous; overall, it is uncertain whether the observed effects are due to the whole cell population or a specific subset of donor MSCs.

Human MSCs (hMSCs) or human MPCs need to be purified according to guidelines for the conduct of clinical trials for SCI (Fawcett et al., 2007; Steeves et al., 2007). These cells should be highly characterized specific donor populations for use in experimental studies aimed toward potential clinical application. The use of autologous, current good manufacturing practice grade donor hMSCs or hMPCs from injured patients (Hodgetts et al., 2013; Sykova et al., 2006) should be preferential in any transplantation therapy.

In a previous study we have shown that a highly purified, multipotent human mesenchymal precursor cell (hMPCs) can be isolated from the bone marrow of SCI patients and transplanted into immunologically deficient (athymic nude) rat hosts following an acute (7 day) SCI; we observed an improvement in functional (behavioral) recovery and promotion of tissue sparing (Hodgetts et al., 2013). All acute SCI becomes chronic with age, and is characterized by large and often multiple cysts within atrophic parenchyma and glial scar tissue (Hodgetts et al., 2008). Chronic contusion injuries in rats are characterized by endogenous repair and scar development that continues for 4 months (Hill et al., 2001). In addition to stimulating axonal growth, other concurrent treatments are needed to diminish the inhibitory environment of the lesion site. Scar tissue that forms after traumatic CNS injury is rich in a variety of proteoglycans, which are upregulated at sites of injury and are inhibitory to axon growth in vivo (Fitch and Silver, 2008). Increasing number of therapeutic agents has been used to improve these inhibitory areas to increase growth in the CNS.

One such molecule is decorin; it is a small, dermatan/chondroitin sulfate proteoglycan of the extracellular matrix surrounding many tissues and is a naturally occurring antagonist of scar formation (Hocking et al., 1998). The structure is a leucine-rich protein core flanked by cystein-rich disulfide groups and either a single dermatan sulfate or chondroitin sulfate proteoglycan GAG chain, both of which are crucial for biological function (Laremore et al., 2010; Seo et al., 2005). Decorin is known to bind and modulate other extracellular matrix molecules such as collagen (Keene et al., 2000); it also suppresses neurocan, brevican, phosphocan and NG2 expression (Davies et al., 2004), which are growth inhibitory ligands associated with scar formation (Sandvig et al., 2004). Mechanistically, decorin is known to mediate biological functions by complex formation with other molecules via the GAG proteoglycan or core protein domains. The wide range of molecules it interacts with can modulate decorin's ability to antagonize or inhibit scar formation, angiogenesis (Neill et al., 2012), inhibitory and proliferative factors, and immunoregulatory molecules (Moreth et al., 2012).

Studies investigating decorin have reported the cellular and molecular environment of acute CNS injuries more supportive of axon growth following decorin treatment (Davies et al., 1999; Logan et al., 1999). Following continuous direct infusion of human recombinant decorin into acute stab injuries of adult rat spinal cord, major reductions in astrogliosis, macrophage accumulation and deposition of axon growth inhibitory Chondroitin Sulphate Proteoglycans (CSPGs) within spinal cord scar tissue were reported (Davies et al., 2004). The use of decorin to reduce scar tissue without the need for invasive surgical debrading is an intriguing molecular approach to improve SCI outcomes.

We hypothesized that decorin administration would improve the inflammatory and matrix environment within the spinal cord for subsequent injection of hMPCs and in turn improve functional and anatomical outcomes achieved by these cells. We have used two decorin isoforms (Core $^{(core)}$ and Proteoglycan $^{(pro)}$), one with and one without a functional GAG core protein (Laremore et al., 2010; Ramamurthy et al., 1996) to further understand decorin activity. This study assessed hMPCs isolated from SCI patients (Hodgetts et al., 2013) that were transplanted as a suspension graft alone or in combination with human recombinant decorin$^{core/pro}$ (Goldoni et al., 2004; Iozzo et al., 2011; Laremore et al., 2010) to improve functional outcomes, by: i) reducing the inhibitory milieu, ii) improving tissue sparing and iii) promoting repair after acute and chronic moderate contusive spinal cord injury (SCI).

Materials and methods

Animals

Adult female CBH-rnu/Arc (athymic nude) rats (120–150 g; Animal Resource Centre, Western Australia) were used in experimental procedures conforming to National Health and Medical Research Council Guidelines (Australia) and approved by the University of Western Australia Animal Ethics Committee. A total of 288 rats were used distributed between 9 experimental groups and 4 time points of analysis.

Isolation of hMPCs

Donor hMPCs were isolated and propagated using methods described previously (Gronthos and Simmons, 1996; Hodgetts et al., 2013; Simmons and Torok-Storb, 1991). These cells were denoted as hMPCs^{GFP} and stored for transplantation experiments. A subsample was also confirmed for Stro-1 expression using FACS at passage number 1 (P1) in vitro.

In vitro culture of hMPCs

Thawed hMPCs^{GFP} not used for transplantation were maintained in αMEM/10%FCS for 24–48 h, in order to determine any neuronal phenotype/marker expression using the panel of antibodies described in the immunohistochemistry section.

Human dermal fibroblasts (hDFs)

Normal human dermal fibroblasts (hDFs) (Lonza #CC-2511) were used as a control cell type for injections after SCI in cohorts of animals ($n = 8$) and also for in vitro culture experiments as a direct comparison for donor hMPCs^{GFP}. All procedures used for hMPC transplantation were reproduced when using donor hDFs.

Co-culture with dorsal root ganglion explants

Dorsal root ganglia dissected from Sprague Dawley rats (embryonic day 15) were prepared as described previously (Plant et al., 2002) and seeded in NLA medium (neurobasal medium plus B27 (both from Invitrogen)) in the center of previously ammoniated Aclar minidishes (Kleitman and Bunge, 1998) that had each been subjected to coating with collagen I and III (Colltech, Australia), and with or without seeding with 5×10^4 early passage (<P3) hMPCs the night before. The explants were left in minimal volume of medium without mechanical disturbance until they had attached. The explants were then flooded with NLA medium containing 40 μg/ml decorin^{core} or decorin^{pro} (Life Cell Corporation), or control (no decorin) and cultured with daily changes of medium for 3 days before fixing and staining with antibody for β-III tubulin (#PRB-435P, Covance, USA, see Analysis of SC sections using immunohistochemistry)