

Promising Advances in Targeted Cellular Based Therapies: Treatment Update in Spinal Cord Injury

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Abstract

Spinal Cord Injury (SCI) affects approximately 10,000 people per year in North America. Over the last twenty years, significant advances have been made in the understanding the pathophysiology of traumatic spinal cord injuries. In addition there have been concurrent advances in embryonic stem cells (ESCs) and the various pathways of their differentiation into neural stem cells (NSCs) and progenitors. It is hoped that these advancing fields can be merged. The administration of ESCs and NSC cells will reconstitute the architecture of the injured spinal cord as well as spinal cord tracts. Thus, this would result in improved anatomical recovery and plasticity allowing for improved neurologic function and locomotion. The authors provide a brief overview of recent publications to illustrate the various approaches to the treatment of SCI with cellular based therapies, including both pluripotent stem cells and neural-committed lineages. Considerable advances have been made in the field. While there is a growing body of laboratory evidence in the literature to support translating cellular therapies into the clinical setting, there are no definitive answers on the efficacy of cellular based therapies in the clinical setting. A variety of cellular therapies have been implemented in novel clinical trials including OECs, fetal-derived NSCs, and Schwann cells. Further refinement of these methods should be made in the future to limit patient morbidity.

Keywords: Spinal cord injury; Stem cells; Neural stem cells; Embryonic stem cells; Neuroplasticity; Cellular replacement strategies; Stem cell transplants

Commentary

Spinal Cord Injury (SCI) affects approximately 10,000 people per year in North America, with a prevalence of over a million people [1]. Over the last twenty years, significant advances have been made in the understanding the pathophysiology of traumatic spinal cord injuries. In addition there have been concurrent advances in adult stem cells and embryonic stem cells (ESCs), and the various pathways of their differentiation into neural stem cells (NSCs) and progenitors. It is hoped that these advances can be combined into an effective treatment strategy. For example, the administration of NSC or progenitor cells could reconstitute the architecture of the injured spinal cord as well as reconnect spinal cord tracts. This would result in anatomical recovery and promote plasticity allowing for improved neurologic function and locomotion. The authors provide a brief overview of recent publications to illustrate the various approaches to the treatment of SCI with cellular therapies, based on multipotent stem cells and neural-committed lineages.

Marrow Stromal Cells

Marrow stromal cells (MSCs) most commonly harvested from the bone marrow, are mesenchymal stem cells because of the capacity for differentiation into tissues of mesodermal origin cells such as bone, fat, muscle, and cartilage [2]. The use of bone-marrow derived stem cells (MSCs) has not resulted in exceptional clinical improvements, nor has its effectiveness in differentiating into NSCs and reconstituting the normal spinal cord architecture been clearly demonstrated [3]. The primary mechanism in promoting recovery is thought to be through its neuroprotective properties. The relative ease of harvesting the necessary volume of cells for therapy, as well as with improvements in less invasive techniques for cell delivery has made this approach an attractive therapeutic option. The technique of arteriography offers an

attractive delivery mechanism for cells over the more established, albeit more invasive, spinal surgery which at the moment carries a higher morbidity. Even though administration of stem cells via arteriography, an outpatient procedure, seems attractive, its efficacy has yet to be proven. At the time of this writing, there are trials registered with the FDA under recruitment or enrollment employing these techniques (Table 1).

Some studies have shown that MSCs can differentiate into NSCs [4,5] as well as express similar markers on MSCs and NSCs [6]. However, experimental evidence demonstrating the differentiation of MSCs into NSCs *in vivo* and post-SCI transplantation by Hofstetter and colleagues [2] is less than definitive because it lacks multiple neuronal markers and physiological evidence. Indeed, the claims of transdifferentiation into neural lineages have been challenged in other studies proposing strict criteria for such evidence [2,7-9].

Nonetheless, the majority of recent clinical trials (Table 1) utilize bone marrow-derived stem cells due to the ease of harvesting and implantation [2,4,10-16]. An additional advantage of using an autologous source of cells for treatment is the ability to forego immunosuppression, which would be needed with use of an allograft (i.e.: cells from another individual). Also, when using MSCs, the yield of cells has been shown to be higher and a shorter interval between

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Author and year	Study design	No. of patients (% male)	Cells harvested	Outcome measured	Summary
Huang et al. 2006 [13]	Case series	16 (88)	Olfactory ensheathing cell (OEC) autograft. Design: Fetal olfactory mucosa grafted into cord lesion. 50 ul suspension containing approx. 1×10^6 cells injected	Phase I study: safety.	Authors conclude OECs are safe in chronic SCI, conclusions limited by Phase I protocol.
Sykova et al., 2006 [15]	Case series	20(80)	Autologous BMSC Design: Cells harvested from posterior iliac crest. Mononuclear cells isolated and infused via transfemoral catheterization of segmental arteries (n=6) or cubital veins (n=14).	One year observation. ASIA scale, Frankel score, SSEP, MEP, MRI evaluation.	Safety demonstrated. Improvement in motor and sensory electrophysiologic measurements was observed within 3 months in 5 of 6 patients with intra-arterial application; Overall, improvement in 5 of 7 acutely injured patients, and in 1 of 13 chronically injured.
Chernykh et al., 2007 [10]	Comparative	36 (72)	Autologous BMSC harvested from iliac crest and engrafted to cord lesion bed in patients with chronic SCI.	Observation time: 9.4 ± 4.6 months. Outcomes measurements: ASIA scale, Barthel, Ashworth score, spinal cord conduction functions, bladder control, and MRI evaluation for safety.	Phase I study. No adverse effects notes clinically or radiographically. ASIA, Barthel, and Ashworth trend towards improvement. No observed effect on other measurements.
Yoon et al., 2007 [16]	Comparative phase I/II trial	53 (79)	Autologous BMSC taken from iliac crest. Mononuclear cells isolated. Cells injected into 6 sites surrounding SCI lesion site. GM-CSF injected subcutaneously once/day for 5 consecutive days each month for 5 months.	Observation: 10.1-11.3 m. Outcomes: Safety, neuropathic pain, AIS grade, MRI evaluation	Safety demonstrated. Overall 30% improvement in AIS grade. Conclusions limited by small sample size.
Mehta et al., 2008 [17]	Case series	180 (NR), 163 with SCI, 17 with indication for other neurological impairments.	Autologous or near-relative with same blood group. Adipose tissue-derived mesenchymal cells, human embryonic stem cell-derived hematopoietic stem cells, and, autologous BM-derived stem cells. 7-10 ml of cells administered into subarachnoid space adjacent to injury site via lumbar puncture.	Observation: 3 m-2 years. Outcomes: Hauser Ambulation Index.	54 of 163(33.1%) improvement in ambulation index. No tumorigenicity. Morbidity (pain, CSF hypotension and spinal headache) from technical difficulties of lumbar catheterization.
Saberi et al., 2008 [21]	Case series	33 (67)	Autologous intramedullary Schwann cells Harvested from (15 cm) sural nerve. Cells were maintained for 3-5 weeks then injected into the injury site through a 3 cm durotomy.	Observation: 2 years. Scales: ASIA, FIM, complications	No significant changes. No complications attributed to procedure.
Cristante et al., 2009 [11]	Case series pilot study	39(72)	Autologous BMSC. Subcutaneous GM-CSF administered for 5 days; day 6: peripheral blood collection via hemodialysis catheter. 2.5×10^6 CD34 positive cells/kg isolated. Cryopreserved and stored for 1 week. Cells delivered with arteriography catheter in right femoral artery access (10 ml/min rate)	Observation: 2.5 years. Outcomes: SSEP.	26 patients with SSEP improvement (66%).
Kumar et al., 2009 [19]	Case series phase I/II trial	297 (NR)	Autologous BMSC harvested from iliac crest. Mononuclear cells isolated by density gradient; Administered into subarachnoid space via lumbar puncture.	Observation: 3 months. Outcomes: Safety. Clinical sensory and motor Observation by neurological exam.	No Adverse outcomes. Conclusions limited by Phase I design.
Pal et al., 2009 [9]	Case series pilot study	30 (NR)	Autologous BMSC harvested from post iliac crest. Monocytes separated by density gradient. Subarachnoid cistern delivery via Lumbar puncture at or below L3.	Observation: 1 year. Outcomes: AIS grade, Barthel index, SSEP, MEP, NCV, MRI.	No adverse outcomes. Unable to demonstrate improvement in outcomes measured.
Kishk et al., 2010 [14]	Case-control	44 (81)	Autologous BMSC harvested from iliac crest. Monocytes separated by density gradient. 5×10^6 to 10×10^6 cells injected via lumbar puncture monthly for 6 months into lumbar cistern.	Observation: 6 months. Outcome: Trunk muscle assessment, VAS pain, Ashworth scale, ASIA scale, AIS grade, bowel and bladder control, SSEP studies	No statistical improvement demonstrated. One case of encephalomyelitis after third injection. 23 patients developed neuropathic pain at site of injection.
Lima et al., 2010 [19]	Case series phase I/II trial	20 (85)	Olfactory mucosa autograft. Mucosa grafted into injury site after laminectomy	Observation: Pts followed for 2 years. Outcome: AIS grade, ASIA motor score, anal sphincter control, bladder sensation, bladder control FIM, WISCI, olfaction recovery	Transient anosmia resolved in all patients. AIS grades improved in 11 of 20.

Seledtsova et al., 2010 [25]	Case series	43 (26)	Fetal neurogenic tissue. Hematopoietic cells harvested from 16-22 week fetal brain and liver tissue. $2-2.5 \times 10^6$ cells given via lumbar puncture. If intramedullary cyst present, cyst drained and cells implanted into cyst cavity. Cells readministered via lumbar puncture 10-14 days after intramedullary implantation for pts with cyst puncture.	Observation: not specified. Outcome: Clinical neurologic improvement	Improvement in clinical exam in 48.9%.
Dai et al., 2013 [12]	Case-control	40 (70)	Autologous BMSC harvested from iliac crest. Passaged cells for 5-7 days. 25 ul (8×10^5 cells/ul) injected lesion cavity.	Pts followed for 6 month. AIS grade, Observation: 6 months. Outcome: ASIA motor score, EMG, paravertebral SSEP, residual urine volume	Statistical improvement in all outcomes measures.
Jiang et al. 2013 [14]	Case series	20 (65)	Autologous BMSC (After weekly passage for 3 weeks, 1×10^6 cells injected via lumbar puncture	Observation: 30 days. Outcome: ASIA scale	Improvement in 15 patients (75%).

BMSC: Bone Marrow-derived Stem Cells, ASIA: American Spinal Injury Association, SSEP: Somatosensory Evoked Potentials, MEP: Motor Evoked Potentials, GM-CSF: Granulocyte Macrophage Colony-Stimulating Factor, FIM: Functional Independence Measure, NCV: nerve conduction velocity, VAS: Visual analog scale, WISCI: Walking Index for Spinal Cord Injury

Table 1: Recent published studies of human stem cell transplantation for spinal cord injury.

harvest and transplantation is required [2]. Chernykh et al. [2] amongst others [11,12,14,17] have obtained autologous bone MSCs from iliac crest marrow, which is an easy procedure with limited morbidity. Clinically when they compared results to controls, a 66% improvement in ASIA, Barthel, and Ashworth aggregate scores was reported.

Yoon²⁷ studied the effects of autologous bone MSCs with the addition of granulocyte macrophage-colony stimulating factor (GM-CSF) with the hypothesis that the inclusion of GM-CSF would improve outcomes by aiding in neuroprotection, stimulate intrinsic NSCs, and inhibit glial scarring. Dividing the treatment arms in this Phase I/II study into acute, sub-acute, and chronic SCI, when compared to controls, they found a lower percentage of distal cord atrophy in the treatment group. Improvements in neurological outcome were observed, but with typical statistical limitations in a Phase I study. Of note, one in five patients in the treatment arm had neuropathic pain, which may be a side effect of using GM-CSF in the spinal cord.

Cristante [3] and coinvestigators treated 39 patients with chronic SCI with MSCs harvested from peripheral blood and then reinfused the cells to the spinal injury segment via arteriography using 2.5×10^6 CD34+ per kg harvested for each patient. This study lacked a control arm; however somatosensory evoked potential (SSEP) recordings over the next 2.5 years showed increased amplitudes.

Overall, bone marrow derived stem cells have been well-studied in phase I trials, with limited morbidity. While limited functional outcome data is available, improvement has been noted in most studies using this cellular source. Results are confounded by small sample size, lack of a control, relatively short term follow-up, and general phase I design.

Olfactory Ensheathing Cells (OECs)

Olfactory ensheathing cells (OECs) are derived from the nasal mucosa, and their natural function is to serve as intermediaries between the peripheral nervous system (PNS) and the central nervous system (CNS). These cells are attractive as transplant tissue since it is believed that they may promote recovery and relaying of information across an injury site. Huang [16] and colleagues harvested OECs from aborted fetuses and, after purification, grafted these cells directly to the SCI lesion cavity. There were no adverse effects in the Phase I results [18-20]. However, these patients required immunosuppression for

the transplant which raises the possibility of morbidity. Alternatively, autograft OECs have been transplanted, with a transient postoperative anosmia. Lima and coinvestigators found a mean improvement in the ASIA impairment scale (AIS) of 2 grades [14]. OECs are an alternative option for transplant consideration. However, they suffer from limitations of graft morbidity and limitations in the small neural cell stock derived from nasal mucosa.

Schwann Cells

Schwann cells can be harvested from a peripheral nerve, such as the sural nerve, or a single intercostal nerve, with limited patient morbidity such as loss of sensation. The purpose of the use of Schwann cells is to replicate their natural function which is to support axonal growth and myelination in the PNS. Saberi [21] demonstrated safe intradural placement of autologous Schwann cells in the post-SCI environment, without outcome data. Further reports on Schwann cell use will be expected upon the conclusion of Phase I studies initiated at major US institutions [22].

Neural Stem Cells

NSCs can be directly harvested from human fetal tissue, or from pluripotent sources such as human embryonic stem cells (ESCs) harvested from a blastocyst or from an induced pluripotent stem cell (iPSC). They are a promising source for cell replacement therapy because of their multipotent properties to generate glial-committed lineage for astrocytes and oligodendrocytes as well as form neurons. Therefore, their applications are being realized in a number of CNS disorders such as ALS [23] and SCI (astrocyte replacement and relay formation, respectively) as well as MS and other demyelinating disorders(oligodendrocyte replacement) [24]. From the aforementioned cellular therapies utilized, NSCs have the capacity to replace lost neural cells, provide neuroprotection, serve as a relay, and provide a scaffold for mediating cellular traffic across the injury cavity.

One case study has reported on the use of harvested fetal NSCs in the chronic SCI environment, with positive results [25]. Based on preclinical data by van Gorp et al. [26] and Lu et al. [27] with the use of human fetal spinal cord derived NSCs, and human embryonic derived NSCs, respectively, Neural Stem Cells, inc. (Rockville, MD), will be entering Phase I of their clinical trial for SCI in 2014. This is the first multicenter trial in the United States involving the transplantation of

a cellular therapy for SCI since The Geron Corporation (Menlo Park, CA) discontinued patient enrollment in 2011 in their trial of human ESC-derived oligodendrocyte-precursor cells (OPC) in SCI. Interest in NSCs as a therapy for SCI is growing, illustrated by a second clinical trial registered by Stem Cells, Inc (Newark, CA) to begin in 2014.

Conclusion

Considerable advances have been made in the understanding of cellular therapies as a mechanism for SCI treatment. There is a growing body of laboratory evidence in the literature to support translating cellular therapies into the clinical setting. This is illustrated by the presence of over 50 registered US clinical trials (<http://clinicaltrials.gov/>). As seen above, a variety of cellular therapies have been implemented in novel clinical trials including OECs, fetal-derived NSCs, and Schwann cells, whose applications are not limited to SCI. Further refinement of these methods should be made in the future to limit patient morbidity. There are a variety of complex pathways and processes involved in the SCI environment, and therefore multiple mechanisms for treatment. It is evident that interest and understanding in this branch of SCI treatment is accelerating.

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