Published in final edited form as:

Spine (Phila Pa 1976). 2009 February 15; 34(4): 328–334. doi:10.1097/BRS.0b013e31819403ce.

GRAFTING OF HUMAN BONE MARROW STROMAL CELLS INTO SPINAL CORD INJURY: A COMPARISON OF DELIVERY METHODS

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Abstract

Study Design—Three groups of 6 rats received subtotal cervical spinal cord hemisections followed with marrow stromal cell (MSC) transplants by lumbar puncture (LP), intravenous delivery (IV) or direct injection into the injury (control). Animals survived for 4 or 21 days.

Objective—Cell therapy is a promising strategy for the treatment of spinal cord injury (SCI). The mode of cell delivery is crucial for the translation to the clinic. Injections directly into the parenchyma may further damage already compromised tissue; therefore, less invasive methods like LP or IV delivery are preferable.

Summary of Background Data—Human bone marrow stromal cells (MSC) are multipotent mesenchymal adult stem cells that have a potential for autologous transplantation, obviating the need for immune suppression. While previous studies have established that MSC can be delivered to the injured spinal cord by both LP and IV, the efficacy of cell delivery has not been directly compared with respect to efficacy of delivery and effects on the host.

Methods—Purified MSC from a human donor were transplanted into the CSF at the lumbar region (LP), into the femoral vein (IV), or directly into the injury (control). After sacrifice, spinal cord sections were analyzed for MSC graft size, tissue sparing, host immune response, and glial scar formation, using specific antibodies as well as Nissl-myelin staining.

Results—LP delivery of MSC to the injured spinal cord is superior to IV delivery. Cell engraftment and tissue sparing were significantly better after LP delivery and host immune response after LP delivery was reduced compared to IV delivery.

Conclusions—LP is an ideal minimally-invasive technique to deliver cellular transplants to the injured spinal cord. It is superior to IV delivery and, together with the potential for autologous transplantation, lends itself for clinical application.

Keywords

intravenous delivery; lumbar puncture; adult stem cells; engraftment efficacy; neuroprotection; immune response

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Introduction

One of the most promising therapeutic approaches for spinal cord injury (SCI) is cellular transplantation^{1, 2}. A number of different cell types have been evaluated, among them adult mesenchymal stem cells derived from bone marrow (MSC). MSC have been shown to promote anatomical and functional recovery in animal models of SCI^{3–9} by promoting tissue sparing^{5, 6}, axonal regeneration⁷ and remyelination^{10, 11}. Therapeutic effects of MSC are primarily due to the secretion of soluble factors and the provision of extracellular matrix that provide protection and support repair. MSC are attractive candidates for transplantation into human patients because they can be easily harvested, expanded and banked, or derived directly from the patient allowing for autologous transplantation, obviating the need for immune suppression¹².

The clinical translation of cellular transplantation strategies requires a safe and efficient means of cellular delivery. In animal models of SCI, the most common delivery is direct injection into the injury site^{5, 13, 14}, which allows a defined number of cells to be delivered, but risks further injuring the cord. Consequently, it may be very difficult to translate to human subjects. Less invasive methods for cell delivery have been investigated, including intravascular delivery^{15–17} (intravenous (IV) and intra-arterial) and delivery into the cerebrospinal fluid^{18–20} (CSF; intra-ventricular and intrathecal). These minimally-invasive techniques decrease the risk to the patient and allow delivery of multiple cell doses over a preplanned time course. A number of ongoing stage I clinical trials utilizing MSC in SCI patients use IV^{16, 21, 22} or intrathecal delivery^{23–25}. Since IV delivery is even less invasive than delivery via CSF by lumbar puncture (LP), it is important to establish the efficacy and safety of both delivery methods. Here, we compare IV and LP delivery of MSC in a rodent SCI model to evaluate engraftment efficiency, tissue sparing, host immune response, and glial scar formation.

Materials and Methods

Cell Preparation

Frozen human MSC at passage 2 were obtained from the Tulane Center for Gene Therapy (New Orleans, LA) from a healthy donor, age 20. Cells were thawed on the day of transplantation and re-suspended in PBS/glucose (Invitrogen, Carlsbad, CA) at 50,000 cells/ μ l. After surgery, a cell sample was re-plated overnight, and viability assessed using Trypan Blue exclusion. Viability was 95% or greater for all cells used for transplants.

Surgical Procedures and Cell Transplantation

All procedures were carried out in accordance with protocols approved by Drexel University College of Medicine's Institutional Animal Care and Use Committee, following NIH guidelines for the Care and Use of Laboratory Animals.

Eighteen female Sprague-Dawley rats (225–250 g; Taconic, Germantown, NY) were immune suppressed with Cyclosporine A (CsA; Sandoz Pharmaceuticals Co.; East Hanover, NJ) administered subcutaneously at 1 mg/100 g/24 h starting 3 days prior to transplantation. All surgeries were performed under anesthesia by intraperitoneal injection of a cocktail of acepromazine maleate (0.7 mg/kg; Fermenta Animal Health Co., Kansas City, MO), ketamine (95 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA), and xylazine (10 mg/kg; Bayer Co., Shawnee Mission, KS).

Subtotal hemisection—All rats received a right subtotal hemisection at cervical level 4–5. A laminectomy was performed and the dura incised above the dorsal root entry zone.

Light aspiration and forceps were used to selectively remove the dorsolateral funiculus. Dura and muscle were sutured and skin closed. Animals were randomly assigned to one of three experimental groups (Table 1).

Lumbar puncture—Animals in the LP group received transplants on post-operative day 1. Animals were anesthetized and LP was performed at lumbar vertebrae L3-5 as previously described^{18, 19}. A 30-gauge needle was used for injection of 1×10^6 cells suspended in 40 μ l PBS-glucose. Cells were injected over a 1-minute period and the syringe was left in place for an additional minute to prevent leakage.

IV delivery—Animals in the IV delivery group received transplants on post-operative day 1. Animals were anesthetized and the femoral artery and vein revealed. 1×10^6 cells in 500 μ l PBS-glucose were injected using a 30-gauge needle over a period of 1 minute. The needle was held in place for an additional minute to prevent leakage.

Direct injection—Acute direct injection is the standard procedure in surgical SCI animal models^{5, 26, 27}. Direct cell injection was performed as previously published^{28, 29} to serve as a standard for cell engraftment and transplant effects on the host. Animals received transplants of 150,000 MSC in 3 μ l PBS-glucose immediately following the injury. The number of cells delivered directly was smaller than in LP or IV delivery because of space limitation at the injury site and limits of cell concentration in the vehicle. In addition, our previous studies suggested that up to 10% of cells delivered by LP accumulate at the site of injury^{18, 20}; therefore, the number of directly injected cells was chosen to approximate the number of cells expected to accumulate at the injury site after LP.

Tissue Processing

Animals were sacrificed at 4 or 21 days following cell transplantation by transcardial perfusion with 0.9% saline, followed by ice-cold 4% paraformaldehyde (Fisher Scientific; Pittsburgh, PA). Spinal cords and major non-neural organs were removed and post-fixed in 4% paraformaldehyde at 4°C for 24 hours, followed by cryoprotection in 30% sucrose (Fisher) at 4°C for 3 days. Tissue was embedded in OCT (Fisher), fast frozen, and stored at -80°C. Tissue was cut in 20 μ m cross sections. Sections were collected on gelatin and poly-L-lysine-coated glass slides and stored at 4°C.

Histology and Immunohistochemistry

Tissue sparing was evaluated from every tenth section stained with Nissl-myelin. To identify graft size, host immune response, and glial scar, every tenth section was immunolabeled with antibodies shown in Table 2. Tissue sections were incubated with primary antibody at 4°C overnight. A biotinylated secondary antibody was applied for 2 hours at room temperature, followed by 2-hour incubation with Vecta Stain ABC Elite reagent kit (Vector Laboratories; Burlingame, CA). After washing with 0.05 M Tris buffered saline (pH 7.6), tissue was incubated with Sigma Fast 3,3'-diaminobenzidine tetrahydrochloride (DAB) tablets to visualize peroxidase reactivity (Sigma; St. Louis, MO). Selected tissue sections incubated with hNuclei antibody were reacted with FITC-conjugated IgG (Jackson ImmunoResearch Labs, Inc., West Grove, PA). Positive and negative controls were routinely performed.

Image Analysis

Images were captured on a Leica DM 5500B microscope with a Retiga SRV camera (QImaging, Surrey, BC, Canada) using SlideBook software (Intelligent Imaging Innovations; Denver, Co). Sections were analyzed using NIH ImageJ software.

To determine graft size and engraftment efficiency, areas positively stained with human nuclei antibody were outlined and the area determined on every 10th section through the lesion using the “Threshold” function in ImageJ. In addition, the total area of the cord was measured and two-dimensional values were obtained. The two-dimensional values obtained were then used to calculate volumes by means of Calvieri’s estimator of volume equation

($V = [\sum (A_1 + A_2 + \dots A_n) \times D] - [A_{max} \times Y]$, where D = Distance between measurements; Y = thickness of each section). The percentage of positively stained graft over total cord volume for each animal within a group was determined using the three dimensional volume values. In the case of direct injection, the graft volume obtained represented 150,000 cells, assuming 100% cell survival after transplantation. To estimate graft volume for direct injection of 1×10^6 cells (the number of cells delivered via LP and IV), we extrapolated the direct graft volume.

On adjacent sections, tissue stained with ED-1 and CD5 for immune cells or GFAP for glial scar was analyzed using the “Threshold” function in ImageJ; volume measures for positively stained tissue were calculated as described above.

Tissue sparing was determined on every 10th section by tracing the entire area of the cord as well as the injury area, obtaining two-dimensional values. Volume measures were calculated from the area measurements as described above to determine the percentage of tissue sparing for each animal within a group. The percentage values were used to make statistical comparisons amongst experimental groups. All values are presented as means of percentage of volume + standard error (SE). Statistical analysis was performed using a one-tailed independent samples t-Test assuming equal variance. Power analysis was performed and confirmed adequate sample size for large effect sizes (Power = 75% – 100%).

Results

LP delivery provides efficient cell engraftment

Cell engraftment is the most important indicator of efficient delivery. To determine engraftment efficiencies after delivery of 1 million cells via LP or IV, sections were stained with antibodies against human nuclei and quantified. Injection of 150,000 MSC directly into the site of injury was used as a reference. Cell engraftment was most efficient after direct injection, since there is no loss on the delivery route (data not shown). We estimated values for the direct transplantation of 1×10^6 cells as described above and obtained relative engraftment values (percentages of total cord volume) of 8.4% at 4 days and 6.1% at 21 days for directly injected cells. Engraftment volumes after LP delivery were 4.1% at 4 days and 3.4% at 21 days. Engraftment efficiency was lowest after IV delivery, with engraftment volumes of 2.3% at 4 days and 1.6% at 21 days. Thus, MSC engrafted more efficiently at the site of injury after LP delivery compared to IV delivery ($p < 0.005$ at 4 days and $p < 0.05$ at 21 days) (Figure 1A, B, C). Graft sizes decreased approximately 20 – 30% between 4 and 21 days in all cases. All lesion sites also contained myelin debris, macrophages and infiltrating cells like Schwann cells or meningeal fibroblasts, in addition to MSC.

Cells injected via LP were localized to the lesion site in the dorsolateral and dorsomedial white matter. No cells engrafted in the spinal cord rostral or caudal of the injury or in the brain. Also, no cells were found in any of the major non-neural organs examined (heart, lungs, liver, and kidney; data not shown). After IV delivery, cells engrafted in similar locations as those cells delivered via LP. Surprisingly, no cells were found in any of the major organs examined (heart, lungs, liver, and kidney; data not shown). Despite the difference in efficacy, location of transplants delivered via LP or IV was comparable to cell engraftment after direct injection.

LP-delivered cells promote early tissue sparing and less prominent glial scarring

Cell transplants have been shown to provide neuroprotection at the site of injury. Spared tissue was measured to evaluate neuroprotective effects of cell grafts after LP and IV delivery. Analysis at the injury site revealed increased tissue sparing following LP delivery compared to IV-delivered cells at the early time point (4 day) with values of 92.7% and 85.3% ($p<0.05$), respectively (Figure 2A, C, D). Direct injection of cells spared 88.1% of the spinal cord tissue (data not shown). Analysis of tissue from animals sacrificed after 21 days showed no significant differences in tissue sparing with 94.0% spared tissue in LP- and IV-treated groups and 95.0% spared tissue in directly injected animals (Figure 2A). The apparent increase in tissue sparing in the IV delivery group was peculiar; however, most likely due to technical issues. Determining the exact injury border becomes harder at later time points when parts of the injury site not occupied with grafted cells are infiltrated with host cells, blurring the border between injury and healthy tissue.

Sections were stained with antibodies against GFAP to determine effects on glial scar formation. We found that the glial scar outlining the injury border was less pronounced after cell delivery via LP compared to IV at 4 days; glial scar volumes at day 4 were 1.1% for LP-treated animals and 2.2% for IV-treated animals ($p<0.01$) (Figure 2B). Cells injected directly had intermediate effects on glial scar formation with a value of 1.5% at 4 days. In animals sacrificed 21 days after treatment, no significant differences were seen in glial scar volume; values of 1.0% (LP), 1.3% (IV), and 1.3% (direct) were obtained (Figure 2B).

LP-delivered cells induce less host immune response

Host macrophage/microglia response in and around the site of injury was slightly lower after 4 days in animals with MSC transplants delivered via LP (8.3%) compared to IV (12.1%) ($p<0.06$) (Figure 3A, C, D). As expected, direct injection of cells, which results in additional injury, induced the highest host macrophage response (14.1%) at the early time point. After 21 days, the host macrophage/microglia response was comparable for all three delivery methods (LP: 3.5%, IV: 4.2% and direct: 3.8%) (Figure 3A).

The host T cell response was minor, as expected since all animals were immune suppressed. However, 4 days after transplantation, the presence of T cells was still significantly lower in response to cells transplanted via LP (0.15%) compared to IV (0.69%) ($p<0.05$) (Figure 3B). Direct injection of cells resulted in T cell influx at 0.42%. After 21 days, the host T-cell response was comparable amongst groups: LP (0.41%), IV (0.45%), and direct (0.45%) (Figure 3B).

Discussion

Cellular transplantation strategies offer great promise to patients with SCI¹. We have previously shown that MSC can serve as a cellular scaffold, secreting neurotrophins for neuroprotection and extracellular matrix supporting axon growth^{5, 29}. Use of MSC as cellular scaffold is particularly attractive given the ease of MSC harvest, the ability for rapid expansion in vitro and the potential for autologous transplantation. However, the optimal method of delivering MSC to patients with SCI has yet to be determined. While direct injection allows guaranteed delivery of MSC to the injury site, it also requires a major neurosurgical procedure with the inherent risk of neurological deterioration. Included in this is the risk from anesthesia, which is not insignificant in patients with SCI³⁰. Furthermore, the patient must undergo a laminectomy to remove bone to access the spinal cord. These laminectomies may increase the patients' future risk for spinal deformity³¹. Often, SCI patients have sustained both bony and ligamentous injury which distorts the anatomy, hence increasing the complexity of obtaining exposure. After bone removal, the dura is opened

over the injury site. The dura is often compromised rendering the patient more susceptible to CSF leakage following surgery. Scarring and inflammation occur both at the level of the dura and the spinal cord tissue. Thus, adequate exposure is difficult to attain, and neural tissue may be contused and friable. Blood products persist along with potential adhesions and cystic changes. This increases the complexity of determining the optimal location for the injection. Even after cells are injected, closure may be difficult secondary to dural defects. Obtaining a water-tight closure is important to prevent leakage of the cellular transplant. These difficulties would increase with each subsequent injection as more scarring will develop. Postoperatively, the incidence of deep venous thrombosis and pulmonary complications are higher in patients with SCI^{32, 33}. Since it is likely cellular transplantation strategies would require multiple injections, these risks may be unacceptable for many patients. This difficulty in translation will limit clinical trials at least initially to patients with complete SCI who cannot show further deterioration, but are also least likely to show significant benefit from transplantation therapies³⁴.

LP and intravascular delivery of MSC offer alternatives to direct injection. Intravascular delivery includes both arterial and IV delivery, although the former is precluded by the multi-segmented arterial supply of the spinal cord, which would require risky cannulation procedures. IV delivery offers the advantages of easy and safe delivery of large cell numbers, but raises additional concerns. One is that circulating cells can be trapped in other organs as a first pass effect as has been observed previously after IV delivery to myocardial infarction^{35, 36}. This problem was not evident in our screen of major organs, possibly because the rat immune system had already removed the human cells in organs that are less immune-privileged than the CNS. In general, IV-injected MSC are likely to be vulnerable to immune cells circulating in the blood. The partial elimination of MSC by the host immune system while in the blood stream is one explanation for the lower engraftment efficiency of MSC at the site of SCI. Others have compared direct and IV delivery of labeled MSC and, comparable to our results, have reported markedly decreased engraftment efficiencies with the latter³⁷. The presence of foreign cells in the bloodstream may also result in a systemically increased immune response which could extend into the spinal cord resulting in elevated presence of macrophages and T cells in IV-treated animals. Signals from these immune cells could then lead to increased activation of astrocytes explaining the more prominent glial scar in IV-treated animals.

In contrast, LP eliminates the risks of direct injection and does not have the disadvantages associated with IV delivery. In addition, it is already established in the clinic, minimally invasive and time-efficient. Complications are rare with the most common being a post-LP headache. In patients who receive cell transplants, this infrequent complication is even less likely to occur as minimal fluid is being withdrawn.

We have previously demonstrated that MSC delivered via LP to SCI rats home to the injury site, prevent secondary injury expansion, and survive for up to 6 weeks^{18, 19}. These early animal studies formed the basis for initial human trials utilizing LP to deliver MSC for the treatment of patients with SCI^{23–25}. In this series of experiments, we compared grafting efficiency, neuroprotective effects and effects on host immune response and scar formation after a single LP or IV injection of MSC. Using a single delivery allowed us to compare results to direct injection, which is the usual delivery method in animal studies. A direct injection protocol precludes the ability of multiple deliveries as it requires a neurosurgical procedure with its inherent risks. Thus, clinical trials utilizing direct injection of cellular transplants have utilized a single injection^{38, 39}. In the current report, a single LP injection compared favorably with a single direct injection with respect to neuroprotective effects such as tissue sparing and host immune response. One possible explanation for the early neuroprotective effect is that LP delivery allows faster and more efficient access of the cells

to the injury site, whereas in the case of IV delivery, cells take longer to accumulate at the site of injury and only a small percentage of cells actually reach the injury site. This early protection, however, is critical because neural cell death, particularly death of neurons and oligodendrocytes, starts soon after injury, leading to expansion of the injury. Engraftment efficiency is lower with LP than with direct injection; however, LP delivery enables multiple injections in a yet to be determined optimal protocol which should increase cell engraftment and likely prove at least comparable if not superior (given that it avoids additional injury) to single direct injection. Future experiments will determine optimal LP delivery protocols in larger animal models and extend the outcomes to include functional assessments.

Acknowledgments

Supported by The Shriners Hospital for Children Grant# 8251 and 8570, NIH grant NS049429 and grant 4015 from the Craig H. Neilsen Foundation

We would like to thank Maryla Obrocka for help with cell culture, Dr. Jed Shumsky for help with statistical analysis and Drs. Marion Murray and Tim Himes for helpful comments.

References

1. Murray M. Cellular transplants: steps toward restoration of function in spinal injured animals. *Prog Brain Res.* 2004; 143:133–46. [PubMed: 14653158]
2. Murray M, Fischer I. Transplantation and gene therapy: combined approaches for repair of spinal cord injury. *Neuroscientist.* 2001; 7:28–41. [PubMed: 11486342]
3. Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. *Lancet Neurol.* 2002; 1:92–100. [PubMed: 12849513]
4. Dasari VR, Spomar DG, Cady C, Gujrati M, Rao JS, Dinh DH. Mesenchymal stem cells from rat bone marrow downregulate caspase-3-mediated apoptotic pathway after spinal cord injury in rats. *Neurochem Res.* 2007; 32:2080–93. [PubMed: 17564836]
5. Himes BT, Neuhuber B, Coleman C, et al. Recovery of function following grafting of human bone marrow-derived stromal cells into the injured spinal cord. *Neurorehabil Neural Repair.* 2006; 20:278–96. [PubMed: 16679505]
6. Sheth RN, Manzano G, Li X, Levi AD. Transplantation of human bone marrow-derived stromal cells into the contused spinal cord of nude rats. *J Neurosurg Spine.* 2008; 8:153–62. [PubMed: 18248287]
7. Wu S, Suzuki Y, Ejiri Y, et al. Bone marrow stromal cells enhance differentiation of cocultured neurosphere cells and promote regeneration of injured spinal cord. *J Neurosci Res.* 2003; 72:343–51. [PubMed: 12692901]
8. Zurita M, Vaquero J. Functional recovery in chronic paraplegia after bone marrow stromal cells transplantation. *Neuroreport.* 2004; 15:1105–8. [PubMed: 15129154]
9. Zurita M, Vaquero J. Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation. *Neurosci Lett.* 2006; 402:51–6. [PubMed: 16713677]
10. Akiyama Y, Radtke C, Honmou O, Kocsis JD. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. *Glia.* 2002; 39:229–36. [PubMed: 12203389]
11. Akiyama Y, Radtke C, Kocsis JD. Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. *J Neurosci.* 2002; 22:6623–30. [PubMed: 12151541]
12. Parr AM, Tator CH, Keating A. Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. *Bone Marrow Transplant.* 2007; 40:609–19. [PubMed: 17603514]
13. Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. *J Neurosci.* 2006; 26:3377–89. [PubMed: 16571744]
14. Parr AM, Kulbatski I, Tator CH. Transplantation of adult rat spinal cord stem/progenitor cells for spinal cord injury. *J Neurotrauma.* 2007; 24:835–45. [PubMed: 17518538]

15. Takeuchi H, Natsume A, Wakabayashi T, et al. Intravenously transplanted human neural stem cells migrate to the injured spinal cord in adult mice in an SDF-1- and HGF-dependent manner. *Neurosci Lett*. 2007; 426:69–74. [PubMed: 17884290]
16. Urdzikova L, Jendelova P, Glogarova K, Burian M, Hajek M, Sykova E. Transplantation of bone marrow stem cells as well as mobilization by granulocyte-colony stimulating factor promotes recovery after spinal cord injury in rats. *J Neurotrauma*. 2006; 23:1379–91. [PubMed: 16958589]
17. Vaquero J, Zurita M, Oya S, Santos M. Cell therapy using bone marrow stromal cells in chronic paraplegic rats: systemic or local administration? *Neurosci Lett*. 2006; 398:129–34. [PubMed: 16423458]
18. Bakshi A, Barshinger AL, Swanger SA, et al. Lumbar puncture delivery of bone marrow stromal cells in spinal cord contusion: a novel method for minimally invasive cell transplantation. *J Neurotrauma*. 2006; 23:55–65. [PubMed: 16430372]
19. Bakshi A, Hunter C, Swanger S, Lepore A, Fischer I. Minimally invasive delivery of stem cells for spinal cord injury: advantages of the lumbar puncture technique. *J Neurosurg Spine*. 2004; 1:330–7. [PubMed: 15478372]
20. Lepore AC, Bakshi A, Swanger SA, Rao MS, Fischer I. Neural precursor cells can be delivered into the injured cervical spinal cord by intrathecal injection at the lumbar cord. *Brain Res*. 2005; 1045:206–16. [PubMed: 15910779]
21. Chernykh ER, Stupak VV, Muradov GM, et al. Application of autologous bone marrow stem cells in the therapy of spinal cord injury patients. *Bull Exp Biol Med*. 2007; 143:543–7. [PubMed: 18214319]
22. Sykova E, Homola A, Mazanec R, et al. Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. *Cell Transplant*. 2006; 15:675–87. [PubMed: 17269439]
23. Callera F, de Melo CM. Magnetic resonance tracking of magnetically labeled autologous bone marrow CD34+ cells transplanted into the spinal cord via lumbar puncture technique in patients with chronic spinal cord injury: CD34+ cells' migration into the injured site. *Stem Cells Dev*. 2007; 16:461–6. [PubMed: 17610376]
24. Callera F, do Nascimento RX. Delivery of autologous bone marrow precursor cells into the spinal cord via lumbar puncture technique in patients with spinal cord injury: a preliminary safety study. *Exp Hematol*. 2006; 34:130–1. [PubMed: 16459180]
25. Saito F, Nakatani T, Iwase M, et al. Spinal cord injury treatment with intrathecal autologous bone marrow stromal cell transplantation: the first clinical trial case report. *J Trauma*. 2008; 64:53–9. [PubMed: 18188099]
26. Bunge MB, Pearse DD. Transplantation strategies to promote repair of the injured spinal cord. *J Rehabil Res Dev*. 2003; 40:55–62. [PubMed: 15077649]
27. Hofstetter CP, Schwarz EJ, Hess D, et al. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci U S A*. 2002; 99:2199–204. [PubMed: 11854516]
28. Lepore AC, Fischer I. Lineage-restricted neural precursors survive, migrate, and differentiate following transplantation into the injured adult spinal cord. *Exp Neurol*. 2005; 194:230–42. [PubMed: 15899260]
29. Neuhuber B, Himes BT, Shumsky JS, Gallo G, Fischer I. Axon growth and recovery of function supported by human bone marrow stromal cells in the injured spinal cord exhibit donor variations. *Brain Res*. 2005; 1035:73–85. [PubMed: 15713279]
30. Hambly PR, Martin B. Anaesthesia for chronic spinal cord lesions. *Anaesthesia*. 1998; 53:273–89. [PubMed: 9613273]
31. Deutsch H, Haid RW, Rodts GE, Mummaneni PV. Postlaminectomy cervical deformity. *Neurosurg Focus*. 2003; 15:E5. [PubMed: 15347223]
32. Branco F, Cardenas DD, Svircev JN. Spinal cord injury: a comprehensive review. *Phys Med Rehabil Clin N Am*. 2007; 18:651–79. v. [PubMed: 17967359]
33. Winslow C, Rozovsky J. Effect of spinal cord injury on the respiratory system. *Am J Phys Med Rehabil*. 2003; 82:803–14. [PubMed: 14508412]
34. Burns AS, Lee BS, Ditunno JF Jr, Tessler A. Patient selection for clinical trials: the reliability of the early spinal cord injury examination. *J Neurotrauma*. 2003; 20:477–82. [PubMed: 12803979]

35. Barbash IM, Chouraqui P, Baron J, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation*. 2003; 108:863–8. [PubMed: 12900340]
36. Cheng Z, Liu X, Ou L, et al. Mobilization of Mesenchymal Stem Cells by Granulocyte Colony-stimulating Factor in Rats with Acute Myocardial Infarction. *Cardiovasc Drugs Ther*. 2008
37. de Haro J, Zurita M, Ayllon L, Vaquero J. Detection of ¹¹¹In-oxine-labeled bone marrow stromal cells after intravenous or intralesional administration in chronic paraplegic rats. *Neurosci Lett*. 2005; 377:7–11. [PubMed: 15722177]
38. Knoller N, Auerbach G, Fulga V, et al. Clinical experience using incubated autologous macrophages as a treatment for complete spinal cord injury: phase I study results. *J Neurosurg Spine*. 2005; 3:173–81. [PubMed: 16235699]
39. Yoon SH, Shim YS, Park YH, et al. Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: Phase I/II clinical trial. *Stem Cells*. 2007; 25:2066–73. [PubMed: 17464087]

Figure 1. (A) LP delivery increased graft volume compared to IV at 4 and 21 days; * $p < 0.005$; ** $p < 0.05$. (B and C) Representative micrographs of tissue sections with MSC transplants delivered via IV (B) or LP (C) at 4 days. Dashed outline indicates injury border.

Figure 2.

(A) LP delivery increases tissue sparing compared to IV delivery at 4 days. No difference in tissue sparing was seen at 21 days; * $p < 0.05$; ** $p < 0.01$. (B) Glial scar was more prominent after IV delivery compared to LP delivery at 4 days; no significant difference was seen at 21 days; * $p < 0.05$; ** $p < 0.01$. (C and D) Representative Nissl-myelin stained sections after IV (C) and LP (D) delivery at 4 days. Injury borders are outlined with dashed line.

Figure 3. (A and B) Macrophage/microglia response appeared decreased at 4 days after LP delivery compared to IV; $p < 0.06$. A significantly decreased T-cell response was observed at 4 days after LP delivery compared to IV delivery; $* p < 0.01$. At 21 days, no significant differences in the host immune response were observed. (C and D) Representative sections for IV (C) and LP (D) animals stained with ED-1 are shown. Dashed line indicates injury border.

Table 1

Experimental Groups

Transplantation Method	Survival Time/Group Size	Transplant
Lumbar Puncture Injection (LP)	4 Day/ n=3; 21 Day/ n=3	1×10^6 hMSC in 40 μ l
Intravenous Injection (IV)	4 Day/ n=3; 21 Day/ n=3	1×10^6 hMSC in 500 μ l
Direct Parenchymal Injection	4 Day/ n=3; 21 Day/ n=3	150,000 hMSC in 3 μ l

Table 2

Antibodies

Name	Type	Dilution	Source
Primary Antibodies (1°)			
Human Nuclei	Mouse Monoclonal	1:100	Chemicon
Macrophage Microglia (ED-1)	Mouse Monoclonal	1:100	Serotec
Pan T-cell (CD5)	Mouse Monoclonal	1:100	Serotec
Glial fibrillary acidic protein	Mouse Monoclonal	1:100	Chemicon
Secondary Antibodies (2°)			
FITC-conjugated Goat-antimouse IgG		1:200	Jackson
Biotin-conjugated Horse-antimouse IgG (rat absorbed)		1:200	Vector Labs