
Stem Cell Transplantation for Traumatic Spinal Cord Injury: What Have We Learned from Previous Experience

Elhawary S., Wagih A., Essam B., Tarek I., Aamer M., Ellessy R. M.

Faculty of Medicine, Cairo University, Cairo, Egypt

Email address:

halagabr@yahoo.com

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Abstract: Spinal cord injuries represent one of the most devastating illnesses that can affect the human body. Before the advent of the regenerative medicine era, it was regarded as an untreatable condition. Stem cell plasticity and translational medicine research open a new window of hope for this category of patients. Clinical trials of stem cell therapy for spinal cord injuries are now more than a decade old. However, the diversity of clinical trial design, cell type, dose and route of injection, make it extremely difficult to draw decisions from these previous experience. This review tries to collect as much evidence as we can from previous studies in order to suggest paths for future research in this setting.

Keywords: Stem Cell Therapy, Spinal Cord Injury, Mesenchymal Stem Cells, Regenerative Medicine, Olfactory Ensheathing Cells

1. Introduction

Spinal cord injury (SCI) is one of the most disabling acquired medical conditions affecting thousands of individuals per year. Furlan and coworkers reported a global incidence of SCI of 8.0 to 246.0 cases per million per year(1). The medical, social and psychological sequelae of SCI are enormous, especially that the current medical and supportive management have little impact on the overall quality of life.

Stem cell therapy is an emerging field of medicine which holds great promise for regenerating tissues and organs. The application of stem cell therapy for SCI is a dream that tackled minds of researchers for years now. After more than a decade of stem cell research for neuroregeneration of SCI, we need to put together evidence we gained and re-evaluate future prospectives in this area.

2. Stem Cells

Stem cells are self-renewing unspecialized cells with variable degrees of plasticity (2). Since first identified by Till and McCulloch in 1963, identification of stem cell characteristics has been a point of vast research (3). Stem cells have the ability to proliferate and differentiate. In addition, they possess the very important characteristic of

homing to their place of origin when injected systemically.

Stem cells can be isolated from nearly all body tissues. According to their origin, stem cells are classified into hemopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), adipose derived stem cells (ADSCs), cord blood stem cells, and organ-specific stem cells (4).

Stem cell transplantation for hemopoietic disorders came into practice since 1968 (5). Observations following extra-hematologic regenerative action of injected stem cells in bone marrow transplantation coined the biologic phenomenon of "plasticity" in which stem cells cross lineage boundaries and adopt biologic characteristics of non-hematologic lineages(6). These observations laid the foundations of "regenerative medicine" (7).

Can Stem Cells Differentiate into Neural Lineage?

MSCs, whether bone marrow derived or adipose derived have been shown to differentiate into neuron cells (8-10), Schwann Cells (11-13), neurotrophic factor secreting cells (14-15), and glial cells (16). Researchers have been able to produce mature and functional dopaminergic neurons from induced pluripotent stem cells (iPS) (17, 18) and Wharton jelly derived MSCs (19). Induction of motor neuron differentiation from embryonic stem cell was successful in many laboratories (20). Various stem cell types have been directed to differentiate into all neural cells such as motor neurons, dopaminergic neurons, oligodendrocytes, astrocytes

and microglia (21).

Possible Mechanisms of Stem cell-induced Neurogeneration:

Vast research, both in-vitro and animal experiments, tried to elucidate the mechanism of neuroregenerative action of stem cells. A number of mechanisms have been proposed: transplanted MSCs induce endogenous neural growth (22). MSC modify microenvironment to produce a more inductive one for regeneration. This is through decreasing apoptosis

associated with injury or inflammation. Moreover, MSCs modulated inflammatory response through (23).

Actual transdifferentiation of stem cells into neural lineage, although not the main pathway of regeneration, is another mechanism (24, 25).

Other mechanisms exclusively found with MSCs are immunomodulation (26, 27) gene delivery (28-30) and angiogenesis (31-33).

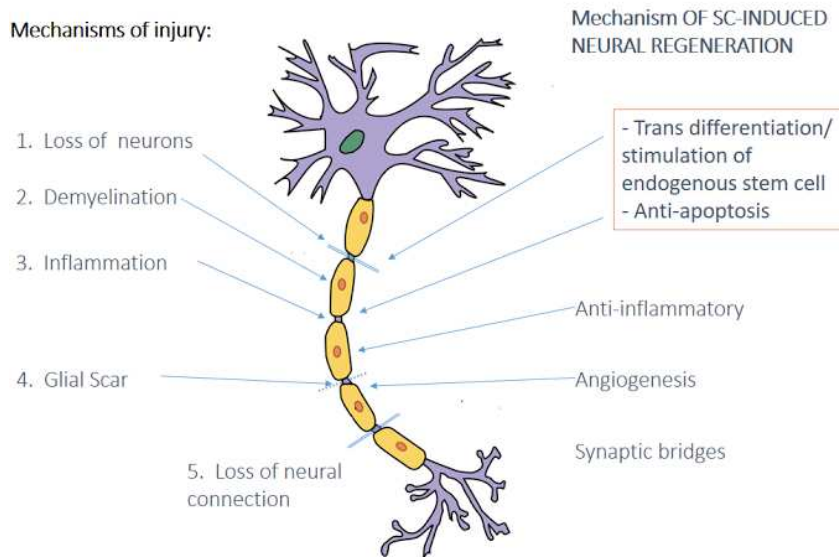


Figure (1). Possible mechanisms of Stem cell-mediated neural recovery after traumatic spinal cord injury.

3. Stem Cell Clinical Trials

Methodology of Research: On searching the website: "www.clinicaltrials.gov" on 19 May, 2015 using the search words "stem cell therapy", we found 4496 studies. When we narrowed the search to "stem cell therapy for spinal cord injuries" we got 33 studies. 6 (18.2%) of them were completed, one (3%) terminated, two (6%) suspended and the other (72.7%) are still recruiting. Search for published clinical trials on pubmed was done using the search words : "stem cells/spinal cord injury" with filters : "clinical trials/human". On google scholar, the search words used were: "stem cells, spinal cord injury AND clinical trials AND human. Eighteen trials were found, three using olfactory ensheathing cells while the others used bone marrow derived stem cells.

The first published trials found dealt with olfactory ensheathing cells : three trials were found published in 2005, 2006, and 2008.

On 2005, Feron et al (34) published a phase I, controlled single-blinded study enrolling 6 patients: 3 serving as cases and 3 serving as control. Olfactory ensheathing cells were obtained through transnasal endoscopic excision from the nasal septum. Cells were then injected into the spinal cord under microscopic control. Dose escalation was done injecting 12, 24 and 48 million cells. Evaluation was done

using radiological assessment for evidence of local complications or tumors, ASIA scale for motor and sensory improvement, mental health assessment, clinical outcome variables (COV) scale for respiratory functions and clinical examination. The authors reported that the procedure was safe and well-tolerated with no reported complications. However, they reported minimal improvement in light touch and pinprick scores but are below clinical detectable levels; with no motor improvement.

In 2006, Lima et al (35) published his trial using olfactory ensheathing cells but grafting them in SCI site surgically, in a pilot safety trial enrolling 7 patients. The authors evaluated improvement using : ASIA score, conventional electromyography, full spinal cord MRI scan, otolaryngological evaluation, CT scan of the nose and paranasal sinuses, psychological assessment, in addition to pre and post-operative urodynamic studies.

The authors reported improvement in ASIA motor scores in all patients, sensory scores in 6 patients and urodynamic studies in 2 patients. They reported that procedure was safe and well-tolerated and recommended further controlled trials.

In 2007, two trials using bone marrow derived cells were published. Yoon and co-workers (36) published a phase I/II controlled, open-label non-randomized trial enrolling 37 patients and 12 controls. The workers used bone- marrow derived mononuclear cell population which includes stem

cells in a dose of 2×10^8 cells after prior stimulation using G-CSF. Cells were injected locally under microscopic control. The study used ASIA scale and motor evoked potential in addition to MRI for follow-up. Mild complications in the form of fever, neuropathic pain and abdominal pain were reported. The efficacy reported was improved ASIA scores in acute and subacute, but not in chronic SCI patients.

Chernykh et al (37) published a phase I controlled trial exploring the safety and efficacy of bone marrow mononuclear cell injection, both locally into the cystic cavity of the spinal cord and intravenously in a dose of 10.9×10^6 . The study evaluated the efficacy using ASIA, Bartel and Ashworth scales. No complications were reported. 12 patients showed sensory improvement, 4 patients showed improvement in motor functions and conduction and 2 patients showed improvement in bladder control.

In 2009, Cristante and colleagues (38) published a prospective non-randomized, non-controlled clinical trial enrolling 39 patients. The study used mobilized bone marrow stem cells collected from the peripheral blood in a dose of 2.5×10^6 leukapheresis-derived mononuclear cells. Patients were followed using somatosensory evoked potential. The results showed positive response in SSEP in 26 patients.

In 2010, three clinical trials were published:

First report of MSC, was in the work of Abo Elkheir et al (39), in 2010 which compared between BM and PB derived MSCs in cases of chronic SCI. The study recruited 150 chronic SCI patients, randomly divided into bone marrow aspiration, stem cell mobilization using G-CSF and control group. Bone marrow group received MSCs in a dose of 2×10^6 /kg in 6 monthly divided doses. Peripheral blood patients received 5 doses of G-CSF and after leukapheresis, received MSCs in the same dose. Patients were followed using ASIA score, trunk motor score and sphincteric control questionnaire. The research team reported a statistically significant mild increase in ASIA score and trunk motor score in patients as compared to controls. Bone marrow group showed slightly more improvement in the motor scores, although not statistically significant. The study reported mild self-limited complications.

Abdelaziz et al (40), in 2010 reported a controlled trial enrolling 30 chronic SCI patients divided as 20 cases and 10 control. The study used autologous bone marrow derived MSCs in a dose of 5×10^6 /kg. Cells were delivered

intraparenchymal and intralesional through open surgery. Two subsequent intrathecal injections were given in two consecutive months. Follow-up was done using clinical assessment using ASIA IMSOP scales, electrophysiology and MRI. The trial reports 30% improvement in ASIA scale A patients, 60% improvement in ASIA scale B patients, with no motor improvement in ASIA scale C patients. Sensory and sphincteric improvement was seen in 7 out of the 20 patients with no correlation to original ASIA scale. The workers reported safety of the procedure with no complications developing on follow-up.

Kishk et al (41) described the results of a case-control series involving chronic SCI patients. The study enrolled 44 patients and 20 controls. Autologous MSCs were injected intrathecally in a dose of $5-10 \times 10^6$ /kg in six divided doses. Patients were followed using ASIA impairment scale, ASIA grading for completeness of injury, Ashworth Spasticity scale, Functional ambulation classification and bladder and bowel control questionnaire. The study reported the improvement in ASIA score and Grade. However, the workers reported complications such as neuropathic pain, increased spasticity and one case who developed encephalomyelitis.

Abo Elkheir et al (42), in 2013 published the result of a registered phase I/II controlled single-blind clinical trial (clinicaltrials.gov identifier: NCT00816803). The study enrolled 70 chronic cervical and thoracic SCI patients with injury durations of at least 6 months were treated with either intrathecal injection of autologous adherent bone marrow cells combined with physical therapy, or with physical therapy alone. Patients were evaluated with ASIA score, electrophysiological somatosensory evoked potential, MRI imaging, and functional independence measurements. The study reported that chronic cervical and thoracic SCI patients treated with autologous adherent bone marrow cells combined with physical therapy showed functional improvements over patients in the control group treated with physical therapy alone, and there were no cell therapy-related side effects. At 18 months post-treatment, 23 of the 50 cell therapy-treated cases (46 percent) showed sustained improvement using the American Spinal Injury Association (ASIA) Impairment Scale (AIS). Compared to those patients with cervical injuries, a higher rate of functional improvement was achieved in thoracic SCI patients with shorter durations of injury and smaller cord lesions.

Table (1). Studies using Stem Cell Transplantation for treatment of Spinal Cord Injuries.

Investigators	Study Design	Cell dose & Type	Mode Of Delivery	Patient diagnosis	Evaluation Scales	Main Results
Elkheir et al, 2013 (42)	I/II Controlled single blind Sample size: 70 patients	Bone marrow derived MSCs	Intrathecal	Chronic spinal cord injury	ASIA score SSEP MRI Functional independence measure	Improvement in 46%
Dai et al, 2013 (43)	Controlled Clinical trial Sample size: 20 study group	BM MSCs 2×10^7	slowly injected to a depth of 3 mm at multiple sites in the central dorsal area across the junction of	Complete and chronic SCI	AIS grading, ASIA score	Improvement in AIS grade And ASIA score in 45%

Investigators	Study Design	Cell dose & Type	Mode Of Delivery	Patient diagnosis	Evaluation Scales	Main Results
Karamouzian et al , 2012 (44)	20 control group		injured and normal spinal cord.			Of patients
	Non randomized clinical trial phase I/II Sample size: 11 study 20 control	7×10^5 to 1.2×10^6 v Bone marrow mononuclear cells	Intrathecal	Fracture dislocation 54.5% Burst fracture 45.5%	ASIA scale	A two-grade Improvement in ASIA scale In 45.5% of patients
Park et al, 2012 (45)	Prospective non-controlled Sample size: 10 patients	MSCs expanded for 4 weeks Dose: 8X10 ⁶ direct injection into the cord 4 X 10 ⁷ into intradural space Intrathecal injection of 5 X 10 ⁷ after 4 and 8 weeks	Direct injection in the cord Injection in Intradural space Intrathecal injection	Chronic spinal cord injury	ASIA scale MRI Electrophysiology	Motor improvement In 6 patients
Gabr et al, 2011 (46)	Prospective non-controlled trial Sample size: 419 patients: 87 cervical, 323 thoracic, 9 lumbar	BM-MSCs 2 X 10 ⁶ /kg On 6 divided monthly doses	Intrathecal	Chronic spinal cord injury	ASIA score Trunk motor score Bowel and Bladder score MRI	43% improvement
Kishk et al, 2010 (41)	Case-control series Sample size: 44 patients 20 control	BM-MSCs 5-10 X10 ⁶	Intrathecal	Chronic spinal cord injury	ASIA scale ASIA score Ashworth scale	Improvement in ASIA score and scale
Elkheir et al , 2010 (39)	Prospective, non-controlled clinical trial Sample size: 419	BM-MSCs PB-MSCs 2 X10 ⁶ /kg	Intrathecal	Chronic spinal cord injury: 87 cervical 323 thoracic 9 lumbar	ASIA score Ashworth scale Trunk motor score SSEP MRI	Improvement: 43.2% overall improvement 40.2% sensory 16% motor 8.6% sphincteric
Abdelaziz et al, 2010 (40)	Controlled prospective Sample size: 20 patients 10 control	BM-MSC 5 X 10 ⁵ /kg	Intralesional followed by 3 monthly intrathecal injections	Chronic spinal cord injury	Clinical ASIA score MRI	Improvement Motor and sensory in 30%
Kumar et al, 2009 (47)	Non randomized noncontrolled clinical trial phase I/II Sample size: 297	unmanipulated bone marrow mononuclear cells	lumbar puncture	Acute and chronic SCI	ASIA protocol	Sensory and motor Improvement in 32.6%
Pal et al 2009 (48)	Clinical pilot trial, uncontrolled Sample size: 30 patients	bone marrow (BM) mesenchymal stromal cells (MSC). expanded ex-vivo dose: 1 x 10 ⁶ cells/kg	lumbar puncture	Thoracic spinal cord injury	ASIA	Safety
Cristante et al,	Prospective	PB leukapheresis-	injected via arteriography	Complete cervical	SSEP	Safety

Investigators	Study Design	Cell dose & Type	Mode Of Delivery	Patient diagnosis	Evaluation Scales	Main Results
2009 (38)	non-randomized clinical trial Sample size: 39 patients	derived mononuclear cells		and thoracic		Improved SSEP sa
Mackay-Sim et al, 2008 (49)	Phase I/II Sample size: 6 patients	OEC	Surgical implantation	Complete thoracic	1. ASIA 2. Functional Indicator measure assessment 3. SSEP 4. MRI	شيفتس safety, one patient showed improvement in improvet من
Subbaiah et al, 2008 (50)	Pilot clinical trial Sample size: 5 patients	BM-MSCs stimulated with brain neutrophic factor	Intrathecal	Chronic spinal cord injury	ASIA score	2 patients improved
Chernykh et al, 2007 (37)	Controlled clinical trial	BMMNCs into cyst and intravenous	Surgical meningo myelo radiculosis and transplatation of cells into cyst and intravenous	Chronic	ASIA Bartel Ashworth	Safety and motor improvement
Yoon et al, 2007 (36)	Phase I/II Open label Non-randomized Controlled Sample size: 35 patients	BM-MNC + GCSF	Intrathecal	Acute and Chronic spinal cord injury	AIS scale Electrophysiology MRI	Improvement in acute, not chronic Increased neuropathic Pain
Lima et al, 2006 (35)	Pilot clinical trial Sample size: 6 patients	OEC	Surgical Implantation	Chronic spinal cord injury	ASIA Scale	2 patients showed improved Sensory and sphincteric Function Improved ASIA score in All patients
Moviglia et al, 2006 (51)	Pilot clinical trial Sample size: 2 patients	MSCS CO-CULTURED with T cells and induced to form neural SC Dose: 5X10 ⁶ -1 X10 ⁹ T cells	Intra-arterial	Chronic spinal cord injury		
Callera and do Noscimento, 2006 (52)	Pilot clinical trial	Autologous BM precursor cells	Injected via lumbar puncture	Chronic spinal cord injury		سسس safety
Feron et al, 2005 (34)	Phase I, controlled, single-blind Sample size: 3 patients 3 control	OEC Dose: 12 X10 ⁶ 24 X10 ⁶ 48 X 10 ⁶	Direct injection into the spinal cord under microscopic control	Chronic spinal cord injury	1. ASIA scale 2. Mental health assessment 3. Clinical outcome variable (COV) scale for respiratory functions	1. Safety 2. Minimal Improvement in Light touch and Pinprick 3. No motor improvement , 2.

4. Technical Considerations

Analysis of data gathered from the previously mentioned trials answers a number of questions and raises other problematic issues. The collective patient population covered

in trials thus far is 976 spinal cord injury patients. This is an adequate sample to draw some conclusions; with careful consideration of the differences in trial settings.

4.1. Type of Study

As regards the type of the study, only four of the published trials were controlled, only two of them were randomized. The other studies were all uncontrolled pilot clinical trials, apart from one case-control series (41).

4.2. Patient Choice

Most trials focused on complete chronic SCI, Kumar et al (47) enrolled both acute and chronic SCI. Moreover, most trials enrolled SCI without subclassification according to injury level. Cristante et al (38) compared cervical and thoracic SCI and Gabr et al (46) compared cervical, thoracic and lumbar lesions.

4.3. Stem Cell Type

The earliest trials used olfactory ensheathing cells as a graft in the injured area of the cord. Although the procedure showed some success, it was an extremely invasive procedure both in obtaining the cells and in transplantation. The minimal improvement did not justify the lengthy and complicated procedure (34, 35, 49).

The next trend used unmanipulated bone marrow mononuclear cells Karamouzian et al (44), Kumar et al (47), ,

Chernykh et al (37), Yoon et al (36), and Callera et al (52); while Cristante et al (38) used leukapheresis derived mononuclear cells. These studies reported variable improvement and short term safety. However, longer safety studies are essential. The possibility of development of autoimmune reaction or sterile inflammatory reaction due to the injection of immunocompetent cells has to be ruled out.

MSCs were introduced as an ideal cell for regenerative medicine, with the advantages of easy isolation, propagation and expansion. Moreover, they were proven to have neuroplasticity, immunomodulatory and positive paracrine action promoting neuroregeneration, Dai et al (43), Pal et al (48), Gabr et al (46), El Kheir et al (39, 42), Kishk et al (41) and Abdelaziz et al (40) used bone marrow derived MSCs. Elkheir et al 2010 compared bone marrow and leukapheresis derived MSCs (39).

To augment the neuroregenerative effect of transplanted MSCs, ex-vivo manipulation can be induced. Pal et al (48) and Park et al (45) used ex-vivo expanded MSCs, Moviglia et al (51) used MSCs induced to transdifferentiate into neural stem cells. Subbaiah et al (50) used brain neurotrophic factor to induce neural differentiation in MSCs.

None of the listed studies compared between unmanipulated and manipulated MSCs.

Table (2). Comparison between various cell types used in clinical trials.

Cells type	Advantages	Disadvantages	Studies
1. OEC	Central nervous system tissue	Complex procedure	Lima et al (35), Feron et al (34), Mackay-Sim et al (49)
2. MNC	Easy isolation	Mixed population Immunocompetent and inflammatory cell contamination	Karamouzian et al (44), Kumar et al (47), Chernykh et al (37), Yoon et al (36), Callera et al (52).
3. MSC	Easy isolation, easy expansion	Very small numbers	Dai et al,(43) Gabr et al, (46), Elkheir et al (39,42), Kishk et al (41), Abdelaziz et al (40)
4. Ex-vivo manipulated MSC	Large number, neural induction	Possibility of contamination and gene mutation	Pal et al (48), Moviglia et al (51), Park et al (45), Subbaiah et al (50)

4.4. Route of Injection

The main routes of stem cell transplantation were surgical implantation, intrathecal injection and systemic intra-arterial injection. Most studies used the intrathecal injection, with reported positive results. The studies using OEC used open surgical intervention Lima et al (35), Mackay-Sim et al (49); while Feron et al (34) used OEC as a cellular graft injected directly into the spinal cord under microscopic guidance. However MSC or MNC implantation using surgical procedure did not report superior results justifying the invasive procedure Abdelaziz et al (40), Chernykh et al (37), Park et al (45). The use of intra-arterial route is limited due to the technical difficulty.

Table 3. Comparison Between Routes of Stem Cell Injection

Route	Advantages	Disadvantages
1. Intrathecal	Technically easy	?BBB, cell loss
2. Intravenous	Cross BBB???	Technical difficulty
3. Intra-spinal	Prevention of cell loss	Invasive procedure

4.5. Cell Dose

Although this is one of the most important issues in stem cell therapy, none of the published clinical trials performed dose escalation study. Cell "dose" estimation is not applicable in OEC grafts as it is not handled as a cellular graft in the studies of Lima et al (35) and Mackay-Sim et al (49). However, Feron et al (34) used cellular OEC. This is the only dose escalation study done, and the reported results showed no correlation between the cell dose and the clinical efficacy. The cell dose used in the published studies varied according to the cell type used. Intrathecally-injected MSCs varied from 1×10^6 /kg used by Pal et al (48) to 4×10^7 used by Park et al (45).

4.6. Method of Evaluation

Evaluation of the efficacy of stem cell therapy is a critical parameter in determining the feasibility of the procedure as well as in comparing variables in cell type, dose, injection regimen and route of transplantation. Objective evaluation

methods are essential to give evidence-based results that can influence the decision making for patients in the future. Clinical evaluation was used by all studies, ranging from disability scales (ASIA scale, AIS scale, Ashworth scale and functional independence measure) to scores such as ASIA score. The importance of these clinical scoring systems is that they reflect the change in quality of life of the patients as a result of improving their independence. This is the most important aspect of therapy. However, the need for biologic and scientific evidence is still needed. Some trials used MRI and SSEP to evaluate the regeneration at the anatomic and functional levels.

In order to design an objective evaluation measure, the mechanism of regenerative action should be known. Up to this point in research, none of the postulated mechanisms of actions has been proven; which make us far from reaching one objective evaluation measure. Until this is finalized, assessment addressing the clinical efficacy is the most acceptable.

4.7. Complications

Apart from self-limited complications related to cell infusion, none of the published clinical trials reported complications due to stem cell therapy. However, longer follow-up is needed to confirm safety of the procedure.

5. Conclusions

After a decade of clinical trials involving stem cell therapy for traumatic spinal cord injury, we can say, on solid ground, that regeneration *can* occur after spinal cord injuries. Clinical trials thus far all documented short term safety and limited efficacy. However, the two important shortcomings are: study design and biologic or physiologic explanations. Challenges for future research are to augment the regenerative effect and verify the exact physiologic mechanism of repair. To reach this, verification of cell type, dose, cell tracking are needed. In addition, construction of randomized controlled trials is essential.

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