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Review Article

Advances in Neural Stem Cell Therapy for Spinal Cord Injury: Safety, Efficacy, and Future Perspectives

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Spinal cord injury (SCI) is a devastating central nervous system injury that leads to severe disabilities in motor and sensory functions, causing significant deterioration in patients' quality of life. Owing to the complexity of SCI pathophysiology, there has been no effective treatment for reversing neural tissue damage and recovering neurological functions. Several novel therapies targeting different stages of pathophysiological mechanisms of SCI have been developed. Among these, treatments using stem cells have great potential for the regeneration of damaged neural tissues. In this review, we have summarized recent preclinical and clinical studies focusing on neural stem cells (NSCs). NSCs are multipotent cells with specific differentiation capabilities for neural lineage. Several preclinical studies have demonstrated the regenerative effects of transplanted NSCs in SCI animal models through both paracrine effects and direct neuronal differentiation, restoring synaptic connectivity and neural networks. Based on the positive results of several preclinical studies, phase I and II clinical trials using NSCs have been performed. Despite several hurdles and issues that need to be addressed in the clinical use of NSCs in patients with SCI, gradual progress in the technical development and therapeutic efficacy of NSCs treatments has enhanced the prospects for cell-based treatments in SCI.

Keywords: Spinal cord injury, Neural stem cells, Clinical trials, Cell-based therapies, Transplantation, Regenerative medicine

INTRODUCTION

Traumatic spinal cord injury (SCI) is a catastrophic event with a high mortality rate and causes physical and emotional difficulties in patients.[1-5] It is defined as injury to the spinal cord, nerve roots, osseous structures, and disco-ligamentous components.[6] The subsequent formation of reactive tissue scarring and cystic cavitation results in the development of molecular and physical barriers to regenerative axonal growth and long-term neurological deficits in SCI. The prevalence and incidence of SCI vary according to geopolitical and economic conditions, and approximately 1,000 new cord injury cases occur every year in South Korea.[7] Although the global incidence is similar between sexes, men have a higher incidence than women aged 20–40 years.[8] Moreover, as the global population tends to grow and health care systems improve, an increase in the absolute number of people living with SCI is expected.[1,3,4]

Anti-inflammatory methylprednisolone sodium succinate is the first-line drug treatment for patients with SCI.[9] After initial management, clinicians surgically decompress the spinal cord and control the lesion site if needed.[10] Many studies have been conducted to prevent or reduce the effects of secondary injury;

among them, research on steroids and neuroprotective alternatives has been discussed for a long time. For the regeneration of damaged neural cells in SCI, various types of stem cells including Schwann cells, olfactory ensheathing cells, embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), and neural stem cells (NSCs), have been examined preclinically and in animal models of SCI.

Recently, transplantation of NSCs has been shown to promote the repair or regeneration of damaged spinal cords. In this review article, we have discussed the characteristics, origin, and recent developments of NSCs in clinical trials of SCI.

BASIC CHARACTERISTIC OF STEM CELLS

Stem cells exhibit 2 characteristics: self-renewal and multipotency. ESCs that are established from fertilized eggs can satisfy the definition of stem cells because ESCs can proliferate indefinitely and differentiate into whole body.[11] Recently, induced pluripotent stem cells (iPSCs) have been suggested to exhibit characteristics similar to those of ESCs.[12] These 2 types of stem cells are called pluripotent stem cells (PSCs). In contrast, adult stem cells (ASCs) reside in organs and regenerate their tissues when damaged.[13] Therefore, ASCs usually have limited lifespan and differentiation potential. In clinical trials to regenerate the damaged central nervous system (CNS), 2 types of ASCs have been used: MSCs and NSCs. The key feature of MSCs is their differentiation potential into mesodermal tissues, such as osteoblasts, adipocytes, chondrocytes, and even other lineages.[14] Moreover, MSCs produce various paracrine factors that have beneficial effects on regeneration and immune modulation.[15] However, several studies have concluded that their beneficial effects are due to functional modulation, and not by direct neuronal regeneration and integration into the injured CNS.[16,17] NSCs are characterized by the expression of typical markers, such as Nestin or Sox2.[18] Generally, they reside in the subventricular zone and the subgranular zone,[19,20] which are specialized niches where young neurons for the olfactory bulb and hippocampus, respectively, are generated.[21] NSCs can self-renew and play a role in neurogenesis in the adult brain.[22,23] NSCs preferentially differentiate into neural lineages such as neurons, astrocytes, and oligodendrocytes, which are attractive for clinical use in CNS diseases.[24] NSCs also secrete beneficial paracrine factors that can help regenerate the damaged CNSs.[25-27] Such characters make NSCs a potent and versatile cellular drug candidate for the treatment of the CNS injuries.

ESTABLISHMENT OF NSCs

NSCs have been established from several sources.[28] Among them, the conventional source of NSCs is the fetal CNS.[8,26,29] Fetal NSCs (fNSCs) have self-renewal potential and neural differentiation capacity.[30] The therapeutic potential of fNSCs has been demonstrated in a model of SCI,[8,29,31] and interestingly, human fNSCs showed neurogenesis after injection into immunodeficient mice *in vivo*.[8,32] fNSCs can differentiate into neurons, which can connect with surrounding neurons.[8,29,31] With promising data from several preclinical studies, most clinical trials have used NSCs derived from the human fetal CNS, including the brain and spinal cord.[33] However, unavoidable ethical issues using fetal CNS are critical for commercial development, and they provide a strong motivation for other cellular sources.

One candidate is the adult NSCs (aNSCs), which can be isolated from the adult CNS. The adult olfactory bulb is the source of NSCs. The olfactory bulb core is an extension of the rostral migratory stream and is thus a potential source of neural progenitors and NSCs.[34] Human spine is another option for aNSCs transplantation.[35-37] Through several preclinical studies, the beneficial effects of aNSCs have been proven in SCI models.[32,38-43] These studies suggested that the beneficial effects of aNSCs come not only from their paracrine effects in neural tissue repair and regeneration, but also from their direct differentiation into various neuronal lineage cells that are integrated and form neuronal networks with the host CNS. This multiple recovery mechanism implies that aNSCs could be an optimal choice in the treatment of SCI.

Despite these advantages, technical difficulties remain to be solved in order to utilize these cells in real-world clinical practice. For the appropriate use of aNSCs, they must be properly isolated and effectively increased in number. Compared with other stem cells, aNSCs reside in relatively restricted areas of the adult CNS.[44] In addition, they have limited and different proliferation capacities according to the lesion type and location.[45] To address the technical difficulties in primary isolation and stable *in vitro* expansion of aNSCs, several research teams have suggested various scientific and technical approaches.[46] Surgical samples from adult CNS are usually very small (1–2 mL) and the number of resident aNSCs within the tissue is also very small. Therefore, aNSCs isolation techniques have been optimized to increase the success rate of primary isolation. First, CNS tissues were physically minced and enzymatically digested into single cells. Enzymatic digestion is a critical step because it

directly affects NSC survival. Papain, trypsin, and collagenase have been commonly used, and in some reports, papain dissociation was suggested to be optimal for the primary isolation of aNSCs.[47,48] After mechanical and enzymatic dissociation of CNS tissues, isolated single cells expand in number. There are 2 alternative culture methods in use: the neurosphere and adherent culture methods. Conventionally, the neurosphere culture method has been used for *in vitro* culture of NSCs.[20,49-57] However, difficulties in stable *in vitro* expansion of aNSCs using suspension culture methods require the development of other culture methods. Moreover, a single neurosphere may not be derived from a single NSC.[58] The possible heterogenic origin of neurospheres could not guarantee the homogeneity of *in vitro*-expanded aNSCs in suspension culture conditions.[59-61] To overcome the limitations of the neurosphere culture method, an alternative adherent culture methods for NSCs, was developed.[52,62-64] In this method, each group used its coating plates to attach NSCs to the plates and various culture medium compositions. Laminin and poly-L-ornithine (PLO) have been used to coat plate frequently, which increase the adherent efficiency of NSCs. To maintain stemness and proliferation of NSCs, the amount of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) have been optimized.[65] For example, we expanded aNSCs from temporal lobectomy samples of epilepsy patients without any neoplastic diseases on PLO-coated dishes in a DMEM/F12 media supplemented with 1% B27, 1% penicillin/streptomycin cocktail, EGF (50 ng/mL), bFGF (50 ng/mL), and 0.5% fetal bovine serum.[62] Using the adherent culture method, aNSCs were expanded *in vitro* from 10^4 to 10^{12} cells within 8 subcultures for 2 months. Moreover, expression of NSC markers such as nestin and SOX2 maintained stably.[62] If the number of aNSCs required for transplantation is 10^7 per patient, at least one hundred thousand patients could be treated with a primary culture of aNSCs.

Recently, technical developments have resulted in the establishment of NSCs from ESCs or iPSCs.[26] When ESCs and iPSCs are induced to differentiate into NSCs by several inducers, such as growth factors and cytokines,[34] these NSCs have similar characteristics to fNSCs, which can induce neurogenesis in the CNS of immunodeficient mice.[66,67] In several preclinical studies, the therapeutic potential of NSCs derived from ESCs or iPSCs has been demonstrated in animal models of SCI.[8,26,29,31] To date, human clinical data using ESCs or iPSCs for SCI treatment are scarce. Only 2 clinical trials (one in each ESCs and iPSCs) are ongoing right now. Compared to the other cellular sources, iPSCs have great advantages in ethical issues and immune rejection.[8] Therefore, interests in NSCs from iPSCs will continuously increase with the advances with iPSCs technology.

PRECLINICAL STUDY OF NSC FOR SCI

Preclinical studies should be designed to address the activity and safety of stem cell-based products for clinical use. Information about the potential mechanism of action of stem cells in the disease indication, the timing of intervention with respect to disease course, and the mode of delivery to the site of action must be investigated in preclinical models. Many preclinical studies using NSCs in animal models of SCI have been reported in the literature,[8,26,29,31] and the therapeutic potential, safety, and several technical aspects of NSCs transplantation have been tested under various conditions.

The characteristics of experimental studies using NSCs are summarized in Table 1. NSCs treatments have been tested at various stages of SCI: acute, subacute and chronic. Mice and rats are the most used animals. In a few studies, human NSCs have been tested in non-human primates.[68-70] The thoracic spinal cord has been the most frequently studied region, where the injury is made by mechanical trauma, such as dropping weight or clip compression. As sudden contusive injury to the cervical spinal cord can be life-threatening, hemitransection is the preferred method for models of cervical SCI. Functional testing scales are somewhat standardized according to animal model species. In mice and rats, the Basso mouse scale or Basso-Beattie-Bresnahan test and CatWalk gait analysis are the most frequently used scales. In addition, many studies have used the von Frey test to evaluate the sensory function. Several studies have also reported functional recovery after transplantation of NSCs as well as graft survival, differentiation and axonal regeneration.[40,42,43,68-81]

Although a number of studies have reported promising results of NSCs treatment in SCI, we still have a long way to go to use NSCs in real-world clinical practice. To move from bench to bedside, determining the differences between the animal model and human SCI and closing the gap caused by inherent limitations of the model should be the first step. In general, there are no reliable animal models that can predict human diseases. Under such circumstances, using a model that most closely represents the critical features of the intended indication is the best alternative. Most human SCI cases are caused by mechanical injury. Consequently, we have developed several animal models of SCI using mechanical trauma to the spinal cord. However, the regenerative potential and physical size of the spinal cord

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Table 1. Summary of preclinical studies using neural stem cells/neural progenitor cells in animal spinal cord injury models in literature

Study	Species	Injury location	SCI model	Transplantation time after SCI	Cell type	Cell source	Route and dose	Combination	Functional evaluation	Result
Pfeifer et al.[84] 2006	Rat	Cervical	Transection	8 Weeks	Auto/allogenic NPCs	Rat brain	Intralesional injection, 2.4×10^5 cells	Fibroblasts	N/A	Showed substantial axonal regeneration
Nomura et al.[104] 2008	Rat	Thoracic	Transection	0 Day	NSCs	Rat brain, spinal cord	Intralesional grafts, N/A for cell dose	Chitosan channel	BBB test	Astrocytic, oligodendrocytic differentiation observed in the channels No functional improvements
Olson et al.[96] 2009	Rat	Thoracic	Transection	0 Day	NSCs	Rat brain	Intralesional grafts, 4.76×10^5 cells	PLGA polymer scaffold	BBB test	Facilitated axonal regeneration No functional recovery
Bozkurt et al.[103] 2010	Rat	Thoracic	Clip compression injury	3 Weeks	NSCs	SC of transgenic rats	Intralesional, 1×10^6 cells	Chitosan channel	BBB test	No functional improvements
Karimi-Abdolrez et al.[41] 2010	Rat	Thoracic	Clip compression injury	6 Weeks	NSCs/NPCs	Mouse fetal brain	Intralesional, 4×10^5 cells	ISD Chondrotinase ABC, EGF, bFGF, PDGF-AA	BBB test Grid walking test Von Frey test	Promoted axonal integrity, plasticity of the corticospinal tract Enhanced the plasticity of descending serotonergic pathways
Kusano et al.[76] 2010	Rat	Thoracic	Clip compression injury	6 Weeks	NPCs	Rat fetal brain	Perilesional injections (4 points around lesion cavity), 2.5×10^5 cells, each	NT-3	BBB test	Enhanced myelin formation Partial improvements of hindlimb motor
Pritchard et al.[69] 2010	Monkey	Thoracic	Hemisection	0 Day	Human NSCs	N/A	Intralesional grafts, 1×10^6 cells	PLGA polymer scaffold ISD	Ambulation chamber video observational neuromotor score	Improvements in postures and movements of leg, foot and toe
Salazar et al.[43] 2010	Mouse	Thoracic	Drop weight Contusion injury	1 Month	Human NSCs	Human fetal brain	Perilesional injections, 5 × cells	None	BMS score, CatWalk test, von Frey test	Differentiation into oligodentrocytes and neurons as well as astrocytes Showed locomotor recovery
Yamane et al.[70] 2010	Monkey	Cervical	Drop weight Contusion injury	9 Days	Human NSCs	Human fetal brain	Perilesional injection, 1×10^6 cells	ISD Galectin-1	Spontaneous movements, Bar grip strength, treadmill test	Motor function improvements
Du et al.[72] 2011	Rat	Thoracic	Transection	0 Day	NSCs	Hippocampus of rat pups	Cord lesion site, cell dose not specified	PLGA scaffold LacZ, NT-3, TrkC gene modification	BBB test Inclined-grid climbing test	Transfected NSCs, co-cultured with scaffold showed the smallest tissue defects at the injury site. Functional improvements observed. Limited ability of corticospinal tract axonal regeneration

(*Continued*)

Table 1. Summary of preclinical studies using neural stem cells/neural progenitor cells in animal spinal cord injury models in literature (Continued)

Study	Species	Injury location	SCI model	Transplantation time after SCI	Cell type	Cell source	Route and dose	Combination	Functional evaluation	Result
Cheng et al.[71] 2012	Rat	Thoracic	Drop weight contusion injury	0 Day	Human NSCs	Human fetal NSCs	Either intrathecal or perilesional SC lesion, 5×10^5 cells	None	BBB test	Functional improvements in both intrathecal and perilesional injections
Lu et al.[42] 2012	Rat	Cervical Thoracic	Hemisection Transection	2 Weeks	Rat and human NSCs	Rat fetal SC Human fetal SC	Intralesional grafts, N/A for cell dose	Fibrin matrices with growth factor cocktail	BBB test	Grafted cells differentiated into multiple cellular phenotypes. Long axon growths with abundant synapses with hos cells Improved motor functions
Amemori et al.[39] 2013	Rat	Thoracic	Balloon-induced compression injury	1 Week	Human NSCs	Human fetal spinal cord	Intralesional, 5×10^5 cells	ISD	BBB, Plantar, walking-beam test	Significant motor, sensory function recovery Showed robust cell survival and partial lesion filling
Kumamaru et al.[102] 2013	Mouse	Thoracic	Drop weight contusion injury	12 Weeks	NSCs/ NPCs	Mouse fetal brain	Perilesional injections (both rostral and caudal side), 5×10^5 cells, each	None	BMS score Grip walk test Footprint analysis	No improvement in motor function
Nemati et al.[68] 2014	Monkey	Thoracic	Drop weight contusion injury	10 Days	NSCs	Monkey brain	Intralesional injection, 1×10^6 cells/kg	None	Tarlov scale and tail movements Limb and tail pinch test	In all scales, transplanted group was faster in recovery.
Salewski et al.[80] 2015	Mouse	Thoracic	Clip compression injury	1 Week	NSCs	Murine embryonal stem cell	Perilesional injections, 5×10^4 cells	ISD	BMS score, CatWalk test, von Frey test	Differentiation to oligodendrocytes Promote remyelination and axonal function Motor function improvements
Cheng et al.[40] 2016	Mouse	N/A	N/A	1 Week	NSCs	Mouse fetal brain	N/A	None	BMS score	Improvements in BMS scores NSC transplantation may modulate SCI-induced inflammatory responses.
Cheng et al.[98] 2016	Rat	Thoracic	Drop weight contusion injury	4 Weeks	Human NSCs	Human fetal NSCs	Either intrathecal or perilesional SC lesion, 5×10^5 cells	None	BBB test	Functional improvement in intrathecal group No functional improvements in perilesional injection group
Jin et al.[74] 2016	Rat	Thoracic	Drop weight contusion injury	13 Weeks	NPCs	SC of transgenic rats	Intralesional & rostral and caudal perilesional injections, 1×10^5 cells each	ISD Chondrotinase Neurotrophic factors	BBB test Grid test Von Frey test Bladder function test	Similar functional improvements between the treatment groups Rats treated with NPC with chondrotinase and neurotrophins showed the most significant improvements in bladder function.

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Table 1. Summary of preclinical studies using neural stem cells/neural progenitor cells in animal spinal cord injury models in literature (Continued)

Study	Species	Injury location	SCI model	Transplantation time after SCI	Cell type	Cell source	Route and dose	Combination	Functional evaluation	Result
Kadoya et al.[75] 2016	Rat Mouse	Thoracic Cervical	Transection	2 Weeks	NPCs	SC of rat and mouse	Intralesional, $1–2\times10^6$ cells	None	Staircase task	Cell graft survived Positive axonal corticospinal tract regeneration and functional synaptic formation Improved forelimb function
Tashiro et al.[81] 2016	Mouse	Thoracic	Drop weight Contusion injury	7 Weeks	NSCs/ NPCs	Mouse fetal brain	Intralesional injection, 5×10^5 cells	Treadmill training	BMS score, von Frey test, Hargreeves plantar test	Improved motor and sensory functions
Lu et al.[77] 2017	Rat	Cervical	Hemisection	2 Weeks	Human NSCs	Human ESCs	Perilesional injections (6 points around lesion cavity), 2.5×10^5 cells, each	Growth factor cocktail	Forepaw placements on grid-walk task	More than a year later, forelimb motor function improved and astrocytes migrated to host tissue.
Nguyen et al.[83] 2017	Mouse	Thoracic	Drop weight contusion injury	0 Day	Human NSCs	Human fetal brain	Perilesional injections, 1.875×10^4 cells	Anti-Ly6G IgG2a	CatWalk behavioral test	Showed astroglial differentiation No locomotor improvements
Robinson et al.[87] 2017	Rat	Cervical Thoracic	Hemisection Drop weight Contusion injury	2 Weeks	NPCs	Rat spinal cord	Intralesional injection, 6.25×10^5 cells	4-growth factor cocktail	N/A	Enhanced graft survival, neuronal differentiation Reduction of the lesion sites
Hosseini et al.[73] 2018	Rat	Thoracic	Clip compression injury	3 Days	MSCs/ NSCs	Rat bone marrow/rat fetal brain	Perilesional injection (both rostral and caudal side)	MSCs	BBB test	Most functional improvement in MSCs/NSCs combined treatment group
Nori et al.[89] 2018	Rat	Thoracic	Clip compression injury	7 Weeks	Human NPCs	Human bone marrow somatic cells	Intralesional injection, 4×10^5 cells	Chondrotinase ABC	BBB test, CatWalk behavioral test, von Frey test	Enhanced NPC survival, migration and oligodendrogenic differentiation Promoted synapse preservation, and enhanced myelination of axons Showed functional improvements
Riemann et al.[78] 2018	Rat	Cervical	Clip compression injury	10 Days	NPCs	Rat fetal brain	Perilesional injection, 4 points 1×10^5 cells each	None	BBB test, CatWalk test, Grid walk test	Showed differentiation along the oligodendroglial lineage and long-term survival Reduction in inflammatory cells and markers, apoptosis Showed functional improvements

(*Continued*)

Table 1. Summary of preclinical studies using neural stem cells/neural progenitor cells in animal spinal cord injury models in literature (Continued)

Study	Species	Injury location	SCI model	Transplantation time after SCI	Cell type	Cell source	Route and dose	Combination	Functional evaluation	Result
Rosenzweig et al.[79] 2018	Monkey	Cervical	Hemisection	2 Weeks	Human NSCs	Human embryonic spinal cord	Intralesional, injection, 2×10^7 cells	ISD	Object manipulation, climbing, and over ground manipulation	Graft survival over 9 months Showed axon regeneration with synapse formation Improved forelimb function
Karova et al.[101] 2019	Rat	Thoracic	Balloon-induced compression injury	1 Week	NPCs	Human fetal spinal cord	Intralesional, 5×10^5 cells	ISD	None	TNF-α downregulation, p65 NF-κB inhibition Reduction of glial scar and cavity size
Lien et al.[86] 2019	Rat	Cervical	Hemisection	2 Weeks	Human NSCs	Human ESCs	Perilesional injections (4 points around lesion cavity), 2.5×10^5 cells, each	Growth factor cocktail	None	No neuron migration
Li et al.[109] 2020	Rat	Thoracic	Transection	0 Days	NSCs	Rat fetal brain	Perilesional injections (2 points rostral, caudal to lesion), 5×10^5 cells	Wnt5a transfection	BBB test	Wnt5a-induced NSC differentiate into neurons and promote motor functional and histological recovery
Jevans et al.[90] 2021	Rat	Thoracic	Drop weight contusion injury	3 Days	NSCs	Rat enteric nervous system	Perilesional injection, 1×10^6 cells	Chondrotinase ABC	Horizontal ladder test	Gastrointestinal tract could be a viable option for cell source. Cotreated with Chondrotinase ABC showed highest regenerative effect with modest functional improvement
Xue et al.[110] 2021	Mouse	Thoracic	Transection	0 Day	NSCs	Mouse spinal cord	Cord lesion site	Collagen nerve regeneration scaffolds Apol8 transfection Epothilone D	BMS score	Promotion of neuronal differentiation, synpse formation Improved hindlimb motor function
Liu et al.[111] 2022	Rat	Thoracic	Transection	0 Day	NSCs	Rat fetal brain	Cord lesion site	3D bioprinting sodium innateate/gelatin scaffold OLGs	BBB test	Improved hindlimb motor function Promoted neural regeneration

SCI, spinal cord injury; NPC, neural progenitor cell; N/A, not available; BBB test, Basso-Beattie-Bresnahan test; NSCs, neural stem cells; PLGA, poly-lactico-glycolic acid; SC, spinal cord; BMS, Basso mouse scale; ISD, immunosuppressant drugs; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; ESC, embryonal stem cell; PDGF-AA, platelet-derived growth factor; MSC, mesenchymal stem cell; NT-3, neurotrophin-3; 3D, 3-dimensional; OLG, oligodentrocyte; iPC-NP, induced pleuripotent stem cell derived neural precursor cell.

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differs among species. Considering that our knowledge regarding the pathophysiological mechanism of SCI is limited, there is a need for multiple animal models to properly address the delivery, efficacy, toxicity and tumorigenicity of NSCs in SCI treatments.

STRATEGIES FOR CLINICAL TRANSITION OF NSCs

In addition to the general consideration of clinical transition from preclinical studies, more knowledge of NSCs needs to be elucidated. The key questions that remain unanswered are as follows: (1) What is the optimal timing for treatment? (2) What are the optimal combinatory or supplementary measures for successful treatment? (3) What is the optimal route of administration? (4) How many cells should be transplanted? (5) Which cellular source should be used, with regard to efficacy, utility, and safety?

1. Optimal Timing for NSC Treatment

Since glial scarring is one of the major barriers to axonal growth and reintegration into neural circuits at the lesion site, cell grafting in the acute phase of SCI might be more beneficial than treatment in chronic-phase SCI. Cheng et al.[82] tested 3 different timings of human NSCs injection (acute, subacute, and chronic) in a T10 contusion injury rat model. The subacute group showed more prominent functional improvements than the chronic group, which supports the idea of the early treatment of SCIs. Furthermore, several studies have suggested that NSCs exert beneficial effects by suppressing neuroinflammation.[27,40] These findings imply that NSC transplantation may benefit the acute to subacute phase of SCI, the period during which the most active inflammatory process takes place. However, several studies have reported contradictory findings. Nguyen et al.[83] injected human NSCs into mice immediately after T9 contusive SCI, and the donor cells showed astroglial differentiation near the lesion but failed to produce functional improvements. In contrast, Salazar et al.[43] transplanted human NSCs into mice 1 month after T9 spinal cord contusive injury, and NSC transplanted mice demonstrated significantly improved locomotor recovery. Therefore, it is difficult to determine that which time window would be the most beneficial for transplanting NSCs after SCI, and we need more data for validation. Many preclinical studies have shown that grafted NSCs survive, migrate and integrate into the injured spinal cord, and differentiate into 3 CNS cell lineages. This suggests that data is insufficient to set specific time window for successful NSCs treatment, and more studies are required to verify the effective treatment timing for NSCs therapy.

2. Considered Combinatory or Supplementary Measures for Successful NSC Treatment

Since SCI is a complicated process with multiphasic cellular and molecular responses that vary over time,[8] testing various strategies in patients with different injury time windows and situations is important along with efforts to find the best time window for the treatment of SCI patients. It is clear that a single treatment modality is not effective in SCI treatment. Several combinatory treatments to enhance grafted cell survival, migration, differentiation and axonal regeneration along with functional recovery have been studied. Synergic treatments with neurotrophic factors such as EGF, bFGF, platelet-derived growth factor, and neurotrophin-3 by implanting genes in NSCs.[41,76] In addition, mixing other cells such as fibroblasts or neuroepithelial-like stem cells with transplanted NSCs to enhance structural repair[84,85]; cotreatment with growth factor cocktail[42,77,86,87]; and adding rehabilitation exercise[81] also showed promising results. In chronic-phase SCI, pretreatment with chondrotinase ABC (ChABC) before transplantation of NSCs seemed to unlock scar tissue around the injury sites and produce a microenvironment conducive for NSCs regeneration. Several preclinical studies that have tested the efficacy of ChABC pretreatment in chronic SCI have also shown locomotor improvements.[41,88-90] These studies are in progress. In the future, there is a need to develop a comprehensive protocol by combining effective strategies according to the injury timeline.

One of the most promising combinatory treatments for NSCs is the use of tissue-engineered scaffolds. The use of scaffolds may act as a bridge that fills the lesion gap and helps reconnect and recover neural networks. Several scaffold types have been developed and tested in preclinical studies. Günther et al.[91] reported that anisotropic alginate hydrogel scaffolds promote axonal regrowth and guided regenerated axons. Huang et al.[92] had used similar scaffolds and demonstrated axonal regrowth through these scaffolds in chronic SCI after the lesion scar was removed. In addition, they have shown significant improvements in functional outcomes and electrophysiological conductivity. Nguyen et al.[83] reported 3-dimensional aligned nanofiber-hydrogel scaffolds could be effective. Furthermore, several other types of scaffolds, such as taxol-modified collagen scaffolds,[93] graphene oxide scaffolds,[94] nanostructured composite scaffolds,[95] have shown efficacy in axonal regeneration. Several studies have tested combinatory treatment with NSCs, and several types of scaffolds have reported reductions in lesion cavities, enhanced grafted

cell survival and axonal regeneration, and functional improvements.[42,69] However, the results have not been consistent in other studies.[72,96] Clearly, various types of scaffolds have shown their efficacy in providing anatomical, structural, and histological framework which can guide and promote axonal regeneration. These scaffolds can replace the injured tissue gap, which would not be possible for regenerating axons to pass through and may help the axons to overpass the lesion site. Future studies are required to verify the role of scaffolds in combination with stem cell-based treatments for SCI.

3. Administration Routes

The issue of NSCs administration routes is also a complicated question that needs to be addressed. Three injection routes are possible for SCI treatment and have been tested: intrathecal, intraspinal, and intravenous. As shown in Table 1, most preclinical studies using NSCs used the intraspinal route for cell transplantation. Amemori et al.[97] compared the intrathecal and intraspinal administration routes in an acute contusive SCI model. Both the methods facilitated functional locomotor recovery; however, cell graft survival at the lesion stie was better in the intraspinal injection group, and they concluded that intraspinal transplantation would be more helpful for long-term spinal cord tissue regeneration. Nevertheless, evidence favoring intrathecal injection as an administration route is also available. Cheng et al.[98] transplanted human NSCs into a contusive rat model of SCI both locally and distally, and significant functional recovery was observed in the distally injected group. Most researchers agree that these beneficial effects arise mainly from the paracrine effects of NSCs. Although these administration routes are clearly disadvantageous in terms of direct neuronal differentiation and tissue regeneration, the intrathecal or intravenous route is a more minimally invasive approach than intraspinal injection, and it can be performed much easier in real-world clinical settings, especially for treating patients in the acute stage of SCI. In summary, the most effective administration route for NSCs transplantation seems to be intraspinal injection. More studies to standardize intraspinal injection procedure and verify its efficacy. In addition, there is also a need for seeking the potential utility of intrathecal or intravenous cell injection in SCI treatment.

4. Number of Cells Needed for Transplantation

The number of cells that should be transplanted to obtain a positive result is another unanswered question. Preclinical studies typically provide the basis for determining the starting human dose. The dose of stem cells is dependent on their stability because effective number of stem cells should be maintained before administration. The number of transplanted cells in preclinical studies presented in the literature ranged between 1×10^5 and 4×10^7 cells per kilogram of animal body weight.[99] Referring to Table 1, most preclinical studies NSCs have used approximately 5×10^5 to 1×10^6 cells for intraspinal cell transplantation. Yousefifard et al.[99] suggested that higher cell doses ($> 3 \times 10^6$ cells/kg) are optimal for transplantation. However, a few studies suggest that there is a certain threshold for the number of transplanted stem cells to survive, and there is no correlation between the number of transplanted cells and functional recovery.[26] Further studies are needed to determine the optimal range of transplanted cell numbers, not only in animal models but also in humans.

5. Issues in Cellular Sources of NSCs

Finally, the cellular source that should be used to obtain NSCs is also an important question in stem cell treatment in SCI. Various cellular sources have been tested. Graft survival, neuronal differentiation and functional recovery have been demonstrated in most preclinical studies where allogeneic NSCs from the fetal brain and spinal cord, as well as human NSCs were transplanted in mouse and rat models.[40,75,77,78,81,86,87,100-102] So far, it seems no specific NSC line showed significant comparative advantage over others. This means that all of NSCs from different cellular sources and lineages should be explored further for their efficacy and safety.

Tumorigenicity and immune rejection are the 2 most important concerns regarding cellular sources. In terms of tumorigenicity, there are numerous experimental design parameters to consider, including the choice of animal model, study duration, route of administration, number of cells tested, positive control selection, and the definition of a positive result. The selected animal model should allow sufficient survival of the stem cell product to enable the assessment of potential tumorigenicity. Therefore, immunocompromised rodents are frequently used for this purpose. Likewise, the study duration should be sufficient to permit the detection of potential tumors. Tumorigenicity studies lasting 9–12 months have been requested by regulatory agencies. To date, reports of tumor formation in NSCs treatments in animal models of SCI are scarce. However, Salewski et al.[80] reported that primitive NSCs derived from ESCs could be transformed into teratomas. Tumorigenicity potential may also reside in NSC lineages and should be closely monitored in future studies.

Ready-made NSCs, which are usually obtained from alloge-

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neic brains or spinal cords, have been used in most preclinical studies.[40,41,72,74-78,102-104] This may be due to limitations in time and autologous source tissue to obtain a sufficient number of NSCs for transplantation. Human NSCs were tested in several animal models with promising results.[39,70,71,77,79,80,83,86,98,101] Long-term survival of grafted cells is necessary for locomotor functional recovery.[32] For graft survival, either immunosuppressants were administered after transplantation or nude mice and nude rats were used. Such conditioning for experimental purposes is possible at a preclinical level. However, the use of immunosuppressants in human patients with acute or subacute stage SCI might be risky. SCIs are usually combined with severe multiple traumatic injuries affecting multiple organs and musculoskeletal regions and using immunosuppressants in such conditions poses high risk for sepsis. Transplanted NSCs have paracrine effects which help SCI recovery, even in the absence of graft survival.[25] Nonetheless, considering that grafted cell survival with neuronal differentiation and integration into the host neuronal network would be a favorable long-term outcome, issues regarding immune rejection should be thoroughly studied. The immune rejection issue has brought iPSCs into the spotlight. With the advantage of avoiding ethical issues, autologous iPSCs have become one of the most attractive cell sources for human NSCs. However, a vast number of studies are required to ensure the efficacy, feasibility, and safety of iPSCs in SCI treatment.

CLINICAL TRIALS USING NSCs

In contrast to the relative abundance of preclinical studies on NSCs transplantation in animal models of SCI, clinical trials of NSCs treatment in patients with SCI, which have been published in the literature have been scarce (Table 2). It is encouraging that several studies reported its procedural safety as well as partial success in functional recovery after NSCs transplantation in patients with SCI.[105-108] However, since the number of enrolled patients was small, and only patients in the subacute (within 1 week to 6 months from the injury) and chronic (over 6 months from the injury) phases of SCI were included in most trials, it is quite difficult to conclude therapeutic efficacy of NSCs, especially in acute phase SCI.

The paucity of clinical trials implies difficulty in translation from bench to bedside in SCI research. The fundamental limitation of translational research is the anatomical difference between experimental animal models and humans. In addition, stem cell therapy in animal models had shown inconsistent results regarding functional recovery. The therapeutic potential of NSCs in SCI treatment was observed, but the absence of a certain, reliable modality resulted in numerous exploratory studies that were far from standardization. Consequently, practical questions such as the location and route of cell transplantation, adequate number of transplanted cells, assessment tools and protocols and variability in NSCs generation are still unknown. Furthermore, real-world problems, such as setting a reliable and safe logistic to obtain, store and deliver NSCs for clinical use,

Table 2. Summary of published clinical trials using neural stem cells in spinal cord injury patients in literature

Study	Country	Clinical phase	Injury location	Treatment timing	Cell type	Cell source	Administration route	Results
Moviglia et al.[107] 2009	Argentina	Phase I	Cerivcal/thoracic	Chronic*	Autologous NSCs		Feeding artery infusion	Functional recovery was shown in 5/8 patients.
Shin et al.[108] 2015	South Korea	Phase I/II	Cervical	22–213 days after SCI	hNSPCs	Human fetal brain	Intralesional injection	Partial improvements in sensorimotor function
Ghobrial et al.[105] 2017	USA	Phase II	Cervical/thoracic	At least 4 months after SCI	NSCs (HuCNS-SC)	Human fetal brain	Intralesional injection	Improvements in overall mean functional outcomes measures
Levi et al.[112] 2018	USA	Phase I	Cervical/thoracic	4–24 months after SCI	NSCs (HuCNS-SC)	Human fetal brain	Intralesional injection	A manual injection technique are safe and feasible
Curtis et al.[113] 2018	USA	Phase I	Thoracic	1–2 years after SCI	NSCs (NSI-566)	Human fetal spinal cord	Intralesional injection	Can be transplanted safely
Levi et al.[106] 2019	USA	Phase II	Cervical	4–24 months after SCI	NSCs (HuCNS-SC)	Human fetal brain	Intralesional injection	Motor functional gains in the treated participants

HuCNS-SC, human fetal-derived central nervous system neural stem cell; NSCs, neural stem cells; NSI-566, NSI-566 cell line human spinal-cord-derived neural stem cell; hNSPCs, human neural stem/progenitor cells; SCI, spinal cord injury; USA, United States of America.
*Specific treatment timing after spinal cord injury was not described.

recruiting patients, and running clinical trials, would be expensive. Several ongoing clinical trials have been attempted despite of hurdles mentioned above.[8,29] However, extensive efforts to find major breakthroughs in SCI treatment are still needed.

CONCLUSION

NSCs are self-renewing and multipotent stem cells that can differentiate into neural lineage cells. For the past 2 decades, many preclinical studies have tested efficacy and safety of NSCs in several animal models of SCI. Successful neuronal differentiation, replacing damaged neural tissue, and functional improvement were observed in several studies. In addition, NSCs secrete neurotropic factors that help protect or regenerate injured spinal cord. In preclinical level, transplantation of NSCs has been proved as a promising therapeutic approach for SCI treatment. However, some of clinical trials of NSCs did not show enough efficacy as expected. These results suggest that a need for further assessment, and the exact mechanism by which NSCs transplantation improves outcomes after SCI should be explored further.

For future perspective, further data such as treatment benefits in terms of neuronal regeneration and functional recovery, adjustments in dose and administration period, optimal injection route, safety, and the most promising cell source for obtaining NSCs should be acquired and verified through future studies. Moreover, matching preclinical animal models and human SCI is another major hurdle to overcome. Finally, it is also important to highlight that a single treatment modality alone may not be sufficient to treat SCI. In addition to cellular transplantation, combinatory therapies such as neurotrophic and growth factors, the use of scaffolds, and neurorehabilitation may be necessary. Their optimal combination and efficacy should also be verified in future studies. Despite these uncertainties, numerous preclinical studies and clinical trials have reported promising results with NSCs treatment for SCI. We are convinced that NSCs have a potential to make a major breakthrough in SCI treatment in the near future.

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