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Neurorestoration Induced by Mesenchymal Stem Cells: Potential Therapeutic Mechanisms for Clinical Trials

Jung Hwa Seo[1,2] and Sung-Rae Cho[1,2,3]

[1]Department and Research Institute of Rehabilitation Medicine, Yonsei University College of Medicine, Seoul;
[2]Program of Nano Science and Technology, Yonsei University Graduate School, Seoul;
[3]Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea.

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Corresponding author: Dr. Sung-Rae Cho,
Department of Rehabilitation Medicine,
Yonsei University College of Medicine,
50 Yonsei-ro, Seodaemun-gu,
Seoul 120-752, Korea.
Tel: 82-2-2228-3715, Fax: 82-2-363-2795
E-mail: srcho918@yuhs.ac

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Stem cells are emerging as therapeutic candidates in a variety of diseases because of their multipotent capacities. Among these, mesenchymal stem cells (MSCs) derived from bone marrow, umbilical cord blood or adipose tissue, comprise a population of cells that exhibit extensive proliferative potential and retain the ability to differentiate into multiple tissue-specific lineage cells including osteoblasts, chondrocytes, and adipocytes. MSCs have also been shown to enhance neurological recovery, although the therapeutic effects seem to be derived from an indirect paracrine effect rather than direct cell replacement. MSCs secrete neurotrophic factors, promote endogenous neurogenesis and angiogenesis, encourage synaptic connection and remyelination of damaged axons, decrease apoptosis, and regulate inflammation primarily through paracrine actions. Accordingly, MSCs may prevail as a promising cell source for cell-based therapy in neurological diseases.

Key Words: Mesenchymal stem cells, paracrine effect, cell-based therapy

INTRODUCTION

Stem cells are emerging as therapeutic candidates in a variety of disease because of their multipotent capacities. Embryonic stem cells are pluripotent and can differentiate into all specialized cell types derived from the three embryonic germ layers. Nevertheless, both ethical and technical considerations limit the clinical availability of these cells. Other potential cell sources, especially for central nervous system (CNS) repair, include fetal neural stem cells (NSCs) and neural precursor cells (NPCs). NSCs can be expanded over multiple passages and do not require the recapitulation of early developmental signals to induce neuroectodermal commitment, as they are already neuralized and committed to a CNS cell fate.[1] However, transplantation of fetal NSCs into the adult brain encompasses numerous ethical and scientific hurdles.[1-3] Because of their plastic ability to survive as undifferentiated cells in ectopic perivascular niches,[4] NPCs have been tested in animal models of neurological diseases. These cells also release paracrine factors that foster survival and proliferation of endogenous neural progenitor cells. However, the application of terminal differentiation of NPCs into neural-lineage cells to replace damaged cells remains controversial.[5]

In contrast, adult stem cells, responsible for maintaining the homeostasis of a specific tissue with fewer ethical problems. One of the most extensively studied populations of multipotent adult stem cells is mesenchymal stem cells (MSCs), which are derived from bone marrow (BM). They can also be isolated from other tissues such as umbilical cord blood,[6] synovium,[7] periosteum,[8] peripheral blood,[9] adipose tissue,[10] skeletal muscle,[11] and placental tissue.[12] MSCs are an excellent candidate for cell therapy because they are easily accessible; can be easily isolated and expanded rapidly *in vitro*;[13] are multipotent;[14,15] involve minimal loss of potency;[16,17] form supportive stroma for hematopoiesis and support stem cell engraftment;[18] may not require immune suppression;[19,20] seem to be largely immunologically inert, paving the way for allogeneic transplantation;[21] secrete numerous trophic factors that modulate inflammation and apoptosis.[22] The large body of work that has accumulated since the discovery of human MSCs has convincingly shown that MSCs from diverse sources retain the ability to differentiate into the mesodermal lineage cells including osteoblasts, chondrocytes and adipocytes.[23,24] They also exhibit the ability to differentiate into neurons-like cells,[25] myocytes and skeletal muscle,[23] although there is a lack of definitive evidence as to the functionality of these differentiated cells.[26,27] Nevertheless, MSCs have considerably contributed to tissue repair in myocardial infarction (MI),[28] stroke,[29,30] meniscus injury,[31] and limb ischemia.[32]

In this review, we will discuss the therapeutic mechanisms of MSCs for neurorestoration and neural regeneration. Thereafter, we will review the published reports of clinical trials for a variety of neurological diseases including stroke, traumatic brain injury, spinal cord injury, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS).

THERAPEUTIC MECHANISMS OF MSCs FOR NEURORESTORATION

Secretion of neurotrophic factors

MSCs secrete a variety of cytokines and growth factors that promote endogenous neuronal growth, neurogenesis and angiogenesis, encourage synaptic connection and remyelination of damaged axons, decrease apoptosis, and regulate inflammation primarily through paracrine actions (Fig. 1).

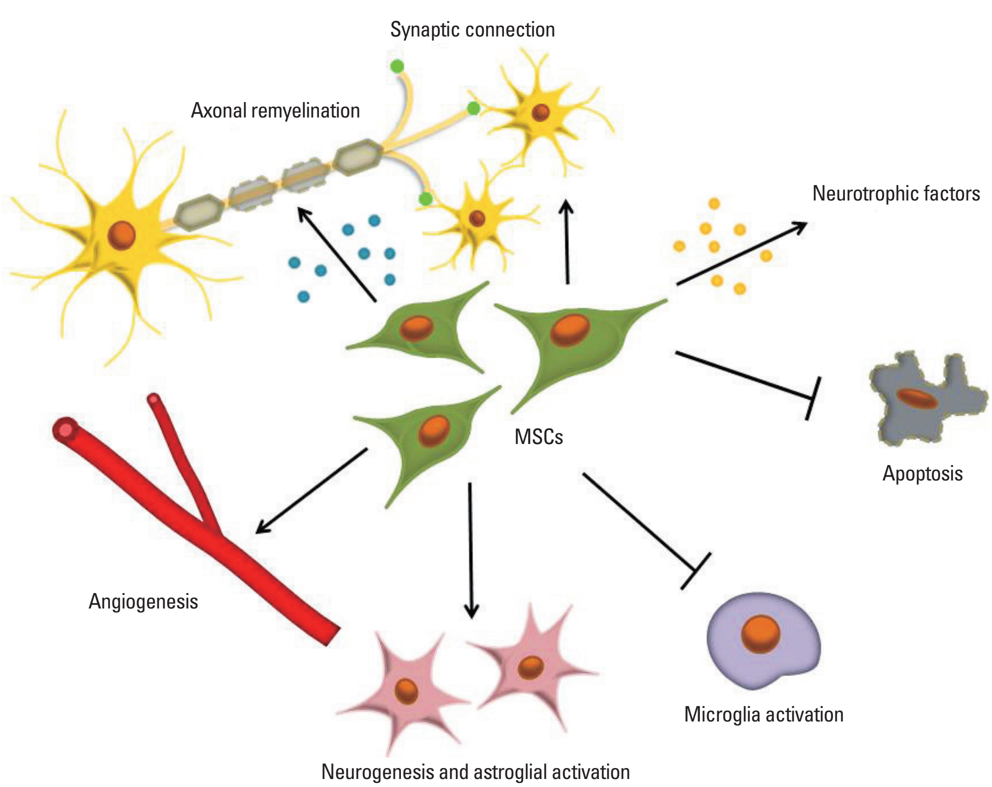

Fig. 1. Potential therapeutic mechanisms of neurorestoration using mesenchymal stem cells. MSCs secrete a variety of neurotrophic factors that promote endogenous neuronal growth, induce angiogenesis, neurogenesis and astroglial activation, encourage synaptic connection and axonal remyelination, decrease apoptosis, and regulate microglial activation primarily through paracrine actions. MSCs, mesenchymal stem cells.

Human MSCs are known to secrete neurotrophic factors including brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF), and nerve growth factor (NGF). After direct transplantation in an animal model of stroke, human MSCs were shown to integrate into host brain, survive, differentiate into neurons and astrocytes, and induce neurobehavioral improvement.[33] BM-derived MSCs can secrete various trophic factors, the secretion of which is enhanced under post-ischemic conditions.[34,35] In our previous study using a rat model of spinal cord injury, neurally induced cells derived from umbilical cord blood also exhibited better functional recovery *in vivo* and secreted more NGFs *in vitro*.[36]

Induction of neurogenesis and astroglial activation

MSCs induce the proliferation of endogenous neural stem/progenitor cells in the subventricular zone (SVZ) and are critical to the survival of newborn cells.[37] They have been shown to be directly involved in neural differentiation after engraftment into damaged tissue and migrate to the CNS to a limited extent.[38] Of particular note, genetically modified MSCs expressing Neurogenin1, a proneuronal gene that directs neural differentiation, increased the therapeutic effects of MSCs in ischemic brain.[39] In addition, MSCs promote the plasticity of damaged neurons and activate astroglial cells to secrete neurotrophins such as BDNF, GDNF and NGF.[34] In an animal model of stroke, intravenous transplantation of BM stromal cells improved functional outcomes by promoting endogenous repair.[29]

Axonal sprouting and synaptic connection

Previous study suggested that extracellular matrix components derived from MSCs can enhance nervous system repair.[40] For example, fibronectin prominently performs essential roles in neuronal survival, axonal sprouting and synaptogenesis following cerebral ischemia.[41] Moreover, extracellular matrix molecules and cell adhesion molecules such as integrin, cadherin, and selectin can promote axonal growth and regeneration.[42]

Anti-apoptotic effect

Reportedly intravenous transplantation of MSCs reduced apoptotic cells stained with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling in an animal model of middle cerebral artery occlusion.[29] This anti-apoptotic effect, together with the previously described capacity to release neurotrophic molecules, may well explain the remarkable functional recovery obtained with the administration of MSCs in experimental models of stroke as well as spinal cord injury.[43,44]

Immunomodulatory effect

MSCs were shown to exert immunomodulatory properties *in vitro*.[45] These features were exploited by researchers in the treatment models of MS and experimental autoimmune encephalomyelitis.[46,47] In PD, MSCs act as neuroprotectors via an anti-inflammatory response to regulate the activity of microglia and to protect dopaminergic neurons.[48,49] In ischemic brain, MSCs were also useful as an immunomodulator.[50] MSCs reduced the numbers of Iba-1^+ and $ED1^+$ inflammatory cells.[39] In our previous study, intravenously transplanted MSCs did not only decrease the level of pro-inflammatory cytokine IL-1β and the proportion of activated microglia, but also increased the level of anti-inflammatory cytokine IL-10, potentially suggesting that early immunomodulation by MSCs was an underlying mechanism of functional recovery in spinal cord injured rats.[51]

Induction of angiogenesis

MSCs secrete a number of growth factors and cytokines, which normally support the proliferation of hematopoietic stem/progenitor cells.[22] In experimental models of cardiovascular diseases such as MI and limb ischemia, the secretion of multiple angiogenic cytokines such as hepatocyte growth factor, basic fibroblast growth factor, insulin-like growth factor 1 and vascular endothelial growth factor was induced from MSCs.[52-56] Existing evidence suggests that these cells can ameliorate ischemic tissue injury, produce appropriate cytokine milieu to promote angiogenesis, and possibly differentiate into endothelial cells.[54,55,57] The evidence seems to point toward the theory that a complex set of trophic factors secreted by MSCs significantly contributes to injury repair *in vivo* by stimulating angiogenesis.

Host cell effects stimulated by MSC transplantation

MSCs are believed to secrete neurotrophic factors, immunomodulatory cytokines, pro-angiogenic factors, extracellular matrix molecules and so on. Thus, the therapeutic effects of transplanted MSCs seem to be derived from paracrine effects rather than direct cell replacement. It is conceivable that MSCs or host brain cells stimulated by grafts may produce such proteins to induce functional recovery and reorganization.[58] MSC-induced secretion of beneficial cytokines by host cells in subjects with neurological diseases such as stroke[29] as well as spinal cord injury[33,44,59] would be a more plausible mechanism given that activation of recipient host cells after cell transplantation has been previously described in other disease models such as MI and ischemic vascular disease.[60,61]

CLINICAL TRIALS IN NEUROLOGICAL DISEASES

Several clinical reports on MSC-based treatments have been published in the past decade, and evoked great excitement for therapeutic candidates for several diseases.[62] As early as the 1990s, cultured MSCs have already been supplemented to reduce acute and chronic graft-versus-host disease among patients receiving allogenic hematopoietic stem cell transplantation, as well as to ameliorate clinical symptoms in osteogenesis imperfecta and glycogen storage disease.[62-64] Currently, the effective therapeutic benefits of MSCs have been supported by increasing numbers of clinical trials on various disorders such as MI, cancer, diabetes mellitus, and Crohn's disease.[65-68]

We will discuss the role of MSCs in neurological diseases, spanning the clinical trials on stroke, spinal cord injury, PD, ALS, and MS. A summary of the cell source, route of delivery, number of patients, study design, dose of transplant, outcome measures and main results for each therapeutic application of MSCs is provided in Table 1.

MSC in stroke and traumatic brain injury

Neuronal and astroglial damages can occur in cerebrovascular disease resulting from the blockage of blood flow in selected brain areas, leading to motor, sensory and cognitive dysfunctions.[3] It has been shown that stroke-induced endogenous neurogenesis and migration of neural stem or progenitor cells into regions of ischemic damage occurs in humans, but the extent to which neurogenesis is able to replace lost neurons or contributes to functional improvement in stroke patients is largely limited.[3,69,70] The limited therapeutic efficacy of endogenous repair processes has encouraged clinicians to incorporate MSCs or BM-derived cells in restorative strategies.

Clinical trials for MSC transplantation to treat stroke and traumatic brain injury are currently ongoing. In patients with middle cerebral artery infarction, the use of autologous MSCs derived from BM has indicated no safety concerns for death, stroke recurrence, or serious adverse events up to 1 year, and trends towards increased functional recovery.[71] This group also reported as a long-term follow-up study for 5 years no serious adverse effects following MSC treatment.[72] Direct administration of MSCs to an injured region following traumatic brain injury has also been performed without adverse events. Briefly, seven patients each received up to 10^9 expanded MSCs as part of a cranial repair operation. The patients were followed up for six months and demonstrated significant improvements in neurological function.[73,74]

MSC in spinal cord injury

Depending on the severity and location of injury, patients present with a varying range of functional impairments, arising from both damage to the local circuitry of the spinal cord and disruption of the ascending and descending fiber tracts.[75,76] All groups who have tested the safety of the transplantation of BM-derived mononuclear cells and stromal cells, or adipose tissue-derived MSCs in patients with spinal cord injury indicate that administration of these cells does not cause any serious adverse effects.[77-80] Geffner, et al.[77] investigated the improvement in quality of life and bladder function without pain or tumor up to 2 years. Syková, et al.[78] reported that five patients who received cells intra-arterially showed improvement up to 1 year.

MSC in parkinson's disease

PD is a progressive neurodegenerative disease whose dopaminergic neurons selectively degenerate in the substantia nigra. Although a variety of drugs such as L-dopa are available, they only remain effective for a certain period in most patients. The limitation of pharmacologic agents increases the need for cell-based therapy as a restorative strategy. In a study recently reported by Li, et al.,[81] two subjects with PD who underwent transplantation of fetal mesencephalic dopaminergic neurons, which had survived for over 10 years, but later developed α-synuclein-positive Lewy bodies in the engrafted donor neurons, suggesting that the disease can propagate from host to graft cells. On the other hand, when autologous BM-derived MSCs were transplanted into the SVZ by stereotaxic surgery, the results suggested the treatment to be safe, and no serious adverse events occurred after transplantation in PD.[82] Additionally, when patients with multiple system atrophy (MSA) were treated with MSCs, greater improvement was noted on the unified MSA rating scale than in untreated control patients, and no delayed adverse effects related to MSC infusion occurred during the

Table 1. A Summary of the Clinical Trials That Used Autologous Mesenchymal Stem Cells in Treatment of Neurological Diseases

	Route of delivery	Number of patients	Study design	Dose of transplant	Main results	Comments	Reference
Stroke	Intravenous	Treatment 5; control 25	Phase 1-2 randomized, controlled safety, efficacy	5×10^{7} cells in two doses	Safety: no death, stroke recurrence, or serious adverse events after 1 year Efficacy: trend towards increased functional recovery	Middle cerebral artery infarcts	Bang, et al.[71]
	Intravenous	Treatment 16; control 36	Phase 1-2 randomized, controlled safety, efficacy	5×10^{7} cells in two doses	Safety: no death, stroke recurrence, or serious adverse events after 5 years Efficacy: trend towards increased functional recovery	Middle cerebral artery infarcts	Lee JS, et al.[72]
Traumatic brain injury	Directly to injured area or intravenous	Treatment 7	Phase 1, open, safety	1st 10^{7}-10^{9} cells 2nd 10^{8}-10^{10} cells	Safety: no death, cell-related serious AE, no toxicity related to MSC within 6 months		Zhang, et al.[73]
Spinal cord injury (SCI)	Intrathecal	Treatment 30	Phase 1-2a, open, safety, efficacy	1×10^{6} cells/kg	Safety: no serious adverse events Efficacy: no change in ASIA scale; improvement in Barthel's index in thoracic injury occurred within 6 months	Patients with complete SCI at cervical or thoracic level	Pal, et al.[79]
	Intravenous	Treatment 8	Phase 1, open, safety	4×10^{8} cells	No serious adverse events	Adipose tissue	Ra, et al.[80]
	Spinal cord, spinal canal, intravenous	Treatment 8 (4 acute, 4 chronic)	Phase 1, open, safety	4×10^{8} mononuclear cells	No tumors or pain, improvement in quality of life and bladder function		Geffner, et al.[77]
	Intra-arterial or intravenous	Treatment 20 (7 acute, 13 chronic)	Phase 1, open, safety	$104\pm55.3\times10^{8}$ mononuclear cells	No complications up to 1 year, 5 patients treated intra-arterially showed improvement	Only 1 chronic SCI reported improvement	Syková, et al.[78]
Parkinson's disease	Intra-ventricle	Treatment 7	Phase 1, open, safety	1×10^{6} cells/kg	Safe and no serious adverse events for 12-36 months.	Feasibility study	Venkataramana, et al.[82]
Amyotrophic lateral sclerosis	Intra-spinal cord	Treatment 10	Phase 1, open, safety	11.4-120×10^{6} cells	Pain (n=7), localized sensory impairment (n=5), localized tingling sensation (n=1)	Feasibility study, two-five injections	Mazzini, et al.[87]
	Intra-spinal cord	Treatment 9	Phase 1, open, safety	57×10^{6} cells	Transient pain (n=4), transient sensory disturbances (n=6)		Mazzini, et al.[86]
	Intrathecal, intrathecal plus intravenous	Treatment 19	Phase 1-2a, open, safety, efficacy	23.4-54.7×10^{6} cells	Safety: no major adverse events Efficacy: neurological disorders unchanged during 6 months	Fever (n=11), headache (n=5), dyspnea (n=1)	Karussis, et al.[85]
Multiple sclerosis (MS)	Intrathecal	Treatment 10	Phase 1-2a, open, safety, efficacy	8.73×10^{6} cells	Safety: iatrogenic meningitis (n=2), headache (n=9) Efficacy: EDSS unchanged (n=4), worsened (n=5), improved (n=1), MRI showed no change (n=7), increased lesion (n=2), decreased lesion (n=1)	Progressive MS with baseline EDSS <6.0 mean follow-up of 19 months	Mohyeddin, et al.[88]
	Intrathecal	Treatment 10	Phase 1-2a, open, safety, efficacy	32-100×10^{6} cells	Safety: encephalopathy, seizure Efficacy: clinical improvement (n=6), worsening of MRI (n= 2)	EDSS 4.0-7.5	Yamout, et al.[89]
	Intrathecal, intrathecal plus intravenous	Treatment 15	Phase 1-2a, open, safety, efficacy	24.5-63.2×10^{6} cells	Safety: fever (n=10), headache (n=10), aseptic meningitis (n=1), no serious adverse events Efficacy: reduction of EDSS	Active MS that did not respond to treatments	Karussis, et al.[85]

MSC, mesenchymal stem cells; AE, adverse event; EDSS, expanded disability status scale.

12-month study period.[83]

MSC in amyotrophic lateral sclerosis

ALS involves a pathology that causes a selective loss of motor neurons leading to a progressive decline in muscle function and poor prognosis. When Nagano, et al. completed a small double-blind clinical trial to assess the effect of intrathecal administration of IGF-1 on disease progression in nine patients with ALS, the high-dose treatment slowed the decline of motor functions, but not bulbar function or vital capacity.[84] On the other hand, both intravenous and intrathecal administration of autologous MSCs were well tolerated, with some preliminary evidence of efficacy in patients with ALS.[85-87] However, large controlled clinical studies are needed to assess possibility for this therapeutic strategy.

MSC in multiple sclerosis

Most phase 1 studies for the safety of MSCs have been conducted in MS.[85,88,89] As a result, Mohyeddin, et al.[88] reported iatrogenic meningitis and headache; Yamout, et al.[89] reported transient encephalopathy and seizure; and Karussis, et al.[85] reported fever, headache and aseptic meningitis. Although serious adverse events related with cell transplantation are likely to be extremely uncommon in MS, the therapeutic efficacy in regards to clinical improvement remains controversial.[85,88,89]

CONCLUSIONS

Experimental evidence in preclinical models of neurological diseases suggests that MSCs are a promising candidate for achieving neural repair and protection. However, current data do not support the possibility that most of the reported effects occur as a result of direct cell replacement. Instead, indirect paracrine mechanisms, including a potent anti-inflammatory capacity, the release of anti-apoptotic and neurotrophic factors, and the ability to induce proliferation of local neural stem/progenitor cells, are likely to promote neurorestoration. Despite tremendous advancements, major unresolved issues concerning therapeutic application still exist. Especially, transplanted MSCs suffer from poor survival and engraftment into host tissue. Further studies are necessary to evaluate in depth the efficacy and safety of MSC-based therapy and whether such treatment would involve a high benefit-to-risk ratio in neurological diseases.

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