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RIVIEW ARTICLE

Current Status of Cell Therapies in Stroke

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Stroke is a leading cause of death and disability in adults. Recovery after stroke is usually limited as there is no definite therapy to restore lost brain function. Cell therapy is an emerging paradigm in stroke therapy for patients with fixed neurologic deficits. Cell therapy for stroke may be greatly different from cell therapy for other disease conditions; the complexity of central nervous system structures and functions may limit its effectiveness. Recently, there have been several clinical trials of cell therapy for patients with ischemic stroke. In this review, the current status and limitations of cell therapy for stroke will be discussed. In addition, recent efforts and perspectives to improve therapeutic efficacy and safety of cell therapy will be summarized.

Keywords: Stem cells, Stroke, Cell therapy, Neurogenesis, Mesenchymal stem cells

Introduction: Position of stem cell therapy in stroke treatment

Stroke is a leading cause of death and the most common cause of physical disability in adults. Compared to other diseases (e.g., cancer), stroke patients usually have a very long morbidity period (from onset to death). The only specific therapies currently available for stroke are intervention to prevent inappropriate coagulation, surgical procedures to repair vascular abnormalities, and thrombolytic therapy. However, thrombolytic treatment can only be applied to certain patients, and various approaches to protect the brain from ischemic damage have met with limited success in clinical practice; consequently, a large proportion of stroke survivors struggle with severe disabilities. To date, relatively little attention has been devoted to developing methods to restore function after ischemic stroke. Although rehabilitation therapy is important to maximize functional recovery in the early stage after stroke, no definite treatment exists to restore lost brain function after stroke. Cell therapy is an emerging paradigm in the stroke treatment field, along with acute recanalization therapy and neuroprotective agents, as a regenerative strategy for patients with fixed neurologic deficits.

This review will focus on the utilization of stem cells in stroke and discuss the current status of this regenerative strategy for patients with ischemic stroke.

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Stem cell mechanisms of action in stroke recovery

Stem cells aid in stroke recovery through various mechanisms of action depending on the specific cell type used. The cell therapy for stroke can be divided into two strategies: cell replacement and enhancing self-repair systems such as endogenous neurogenesis. Transplanted cells could provide trophic support or replace the missing brain cells in the infracted area. Various cell types, including embryonic stem cells, endogenous organ-specific stem and progenitor cells, cell lines, and non-neural adult stem cells, can be used. Ideal candidate cells for transplantation would (1) be autografted (i.e., easy to obtain and culture to get sufficient cell dosages with no need for immune suppression), (2) require minimal manipulation (per FDA recommendations), and (3) have appropriate stem cell characteristics (i.e., self-renewing, non-carcinogenic cells that migrate to injured areas and undergo site-specific differentiation to an appropriate phenotype).

Table 1. Target strategies of cell therapy in various diseases

Conditions	Cell replacement	Enhancement of endogenous recovery process	Neuroprotection	Anti-inflammatory effect
Loss of selective CNS cell-type				
Parkinson's disease	Yes	No	Yes	Limited
Huntington's disease	Yes	No	Yes	Limited
Multiple sclerosis	Yes	Unknown	Unknown	Yes
Pan-necrosis within certain brain regions				
Stroke	No	Yes	Variable*	Yes
Spinal cord injury	No	Yes	Variable*	Yes
Systemic diseases				
Myocardial infarction and limb ischemia	No	Yes†	Variable*	Yes
Skeletal disease	Yes	No	Unknown	Yes

*Possible, if applied at a very early stage. †Mainly, angiogenesis.

The choice of cells for transplantation may depend on the targeted strategic mechanism deemed most beneficial. Even when the same type of cells are transplanted, the beneficial action of transplanted cells may differ depending on disease conditions (1); thus, the choice between different of cell therapies should be based on the disease condition (Table 1).

Cell replacement

Replacement of damaged tissue with exogenous cells is attractive and may be an ideal approach in certain disease conditions. For example, replacement of dopamine-secreting cells can be an ideal approach in patients with Parkinson's disease, in which progressive degeneration of dopamine-secreting neurons is the pathophysiologic problem. For this purpose, embryonic stem cells, neural stem cells, and neuronal-differentiated cell lines can be used. In the stroke field, this cell replacement strategy has several limitations. First, unlike Parkinson's disease, cell types other than neurons and complex neural circuitries are lost in infarcted brain regions; thus, the complexities of central nervous system structure and function may limit the role of this cell replacement strategy in stroke. Restorative therapy for stroke may instead include pharmacological or cellular therapy for synaptogenesis and angiogenesis as well as neurogenesis. Second, although transplanted cells may exert their therapeutic effects by directly replacing missing cells, the cells that differentiate to neurons rarely survive or form functional synapses, especially in toxic conditions such as stroke. Consequently, the role of stem cells in stroke recovery may be more complicated, and enhancing the endogenous recovery system may be more appropriate in this situation. Pre-clinical data shows most cells transplanted die within a few weeks; it is highly unlikely that most transplanted cells integrate into the cerebral tissue and make appropriate connections within days after transplantation (2). Cell replacement can be considered for chronic stroke patients. However, the ethical dilemmas of embryonic stem cell research and the problems associated with allo- and xeno- transplantation limit the clinical use of stem cells for this purpose.

Enhancing self-repair systems

Adult stem cells exist in the brain in small numbers, remaining quiescent (non-dividing) for many years until activated by disease or tissue injury. These neural stem/progenitor cells are located mainly at the subventricular zone lining the lateral ventricles and subgranular zone, part of the dentate gyrus of the hippocampus (3, 4). Preclinical studies show the importance of neurogenesis in animal models of stroke (5-7). Newly-divided cells migrate to the stroke site, express neuronal and glial-specific markers, (8, 9) and form synapses (10). Neuronal progenitor cells migrate long distances in the peri-infarct tissue of stroke patients, showing that neurogenesis is also observed in humans (11). Stroke-induced neurogenesis continues for up to one year (6) even in the aged brain (7). In addition, Dr. Carmichael and his colleagues suggest that there is a 'neurovascular niche' because neurogenesis occurs in close association with endothelial cells and angiogenesis is associated with neurogenesis (12).

However, the capacity for self-repair appears to be limited; about 80% of migrating newly-divided neurons died within 6 weeks, and only about 0.2% of damaged cells were replaced via neurogenesis (8, 13-16). Animal models of normal learning and functional recovery after stroke reveal that repetitive practice, exercise, or an enriched environment can evoke endogenous neurogenesis and expression of signaling molecules such as brain-derived neurotrophic factor (BDNF) (17, 18). PET scans of taxi drivers

provided evidence that learning new things spurs neural growth in humans (19) and there have been numerous efforts to enhance endogenous neurogenesis via pharmacological therapy or stem cell transplantation (20-22). However, pharmacological therapies possess several problems. First, intraventricular administration is needed because blood-brain barrier permeability is low for most growth factors due to high molecular weight and low lipid solubility; additionally, administration of trophic factors can be accompanied by serious systemic adverse effects (23). Transplanted stem cells might enhance endogenous neurogenesis (2, 9, 24, 25). Moreover, stem cells can migrate to the injured area, pass the blood-brain barrier, and secrete trophic factors into the brain.

Adult stem cells (neural or non-neural) may attenuate inflammation, protect against ischemic degeneration, enhance endogenous recovery processes, and replace missing cells (1). Bone marrow-derived mesenchymal stem cells (MSCs) have been most extensively studied. The use of MSCs is attractive in that autologous MSCs could be used, avoiding any immune reaction. The therapeutic window for intravenous MSC administration is at least 1 month after stroke (26). MSCs are thought to have multiple roles (27) (Fig. 1) because they are multi-potent and can trans-differentiate into neural cells (28, 29). Brain samples taken from women who received bone marrow transplants from male donors showed Y chromosome-containing nerve

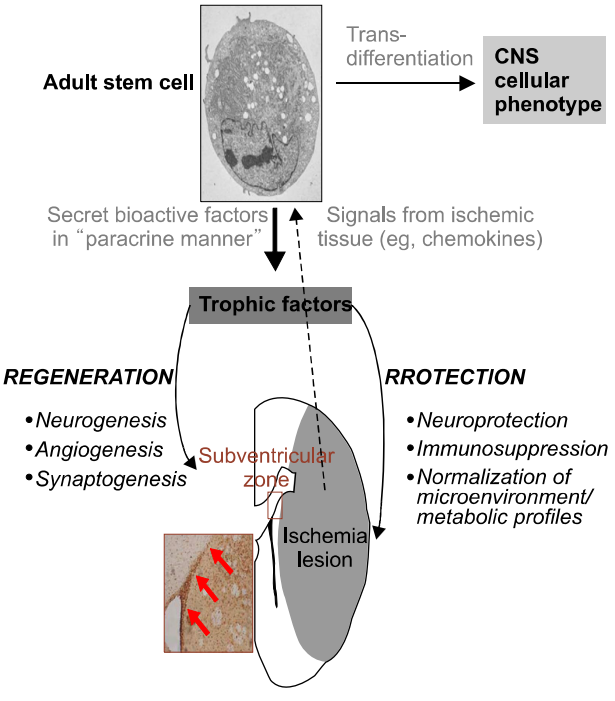

Fig. 1. Stem cell mechanisms of action in stroke recovery.

cells, suggesting MSCs function in the brain (29). Additionally, various trophic factors influence neurogenesis (proliferation, survival, and differentiation of neural progenitor/stem cells) in the mature brain (30), and the capacity to release trophic factors is key to the beneficial effect of MSCs in cerebral ischemia (9, 31, 32). MSCs secrete cytokines and growth and trophic factors, which activate mechanisms such as neurogenesis, angiogenesis, and synaptogenesis to improve neurological function (9, 24, 32, 33). MSCs secrete a variety of bioactive substances such as neurotrophins, interleukins, and stem-cell factors (31, 34). If derived from adult human bone marrow, MSCs secrete trophic factors, including BDNF, GDNF (glial cell line-derived neurotrophic factor), NGF (nerve growth factor), VEGF (vascular endothelial growth factor), and HGF (hepatocyte growth factor) (9, 31, 34-36). Our recent pre-clinical data show that levels of BDNF, VEGF, HGF, NGF, GDNF, and basic fibroblast growth factor (bFGF) were increased in rat brain tissue after intravenous application of human MSCs (9). Recently, Dr. Chopp and his colleagues reported that MSCs promote new circuitry and white matter remodeling as evidenced by the fact that stroke treatment with MSCs created new circuits in the spine as well as the brain (both ipsilateral and contralateral hemispheres) (37).

Inflammation is one of the key mechanisms of ischemic cell death. Stem cells (neural stem cells, hematopoietic stem cells, and umbilical cord blood cells) exert anti-inflammatory reactions via splenic inhibition in stroke models (38-40). Lee and colleagues showed that intravenous administration of neural stem cells blocks inflammatory reactions and brain swelling in a hemorrhagic stroke rat model via splenic inhibition of TNF-alpha secretion (38). They suggested that anti-inflammatory functionality promoted neuroprotection, mainly by interrupting brain-spleen communications that lead to splenic inflammatory responses after stroke (38). In addition, spleen-independent anti-inflammatory mechanisms may exist. Dr. Oh and his colleagues demonstrated that both topical MSC and MSC-conditioned media on chemically injured cornea reduced corneal inflammation, and suggested that these anti-inflammatory actions of MSC might be mediated in part through a paracrine pathway involving soluble factors (41, 42). Our recent study also showed that co-culture of microglia and MSCs decreased microglial activation, TNF-alpha and iNOS mRNA expression, and TNF-alpha protein production (43). These data suggest that MSCs have a neuroprotective effect through anti-inflammatory action mediated by the modulation of microglial activation (43).

Lastly, stem cell therapy may improve local environmental conditions in ischemic regions. Our pre-clinical studies of brain metabolic profiling have shown that intravenous MSC infusion normalized ischemia-induced changes in free fatty acid levels (44).

Clinical trials and the gaps between bench and bed-side

As shown in Table 2, several clinical trials have been performed recently in stroke patients (45-50). They varied in terms of patient characteristics, cell type, and mode of treatment. Results from these pilot trials are challenging but also raise important issues.

First, the selection of candidate patients for cell therapy based on severity and location of lesions and time of application (i.e., chronic vs. acute stage) should be determined. Patients suffering a very severe stroke are likely to have a poor outcome regardless of intervention (51). On the contrary, patients with minor strokes are not suitable for these potentially risky experimental treatments. In addition, patient selection should be performed at an optimal time point (52, 53), and precise prognostic algorithm or a cutoff point for predicting long-term outcome based on data from serial stroke score is needed (53).

Secondly, optimal approaches for cell therapy should be

Table 2. Clinical trials of cell therapy in stroke patients

	Neuronal cells		Neural stem/ progenitor cells	Hematopoietic stem cells	Mesenchymal stem cells	Bone marrow mononuclear cells
Reference	Kondziolka et al. 2000	Kondziolka et al. 2005	Savitz et al. 2005	Sprigg, et al. 2006	Bang et al. 2005	Mendonça et al. 2006
Study design	Phase I Treatment, N=12	Phase II Control, N=4 Treatment, N=14	Phase I Treatment, N=5	Phase IIa Control, N=12 Treatment, N=24	Phase 1-II Control, N=25 Treatment, N=5	Case report Treatment, N=1
Infarct	Chronic Basal ganglia	Chronic Basal ganglia infarct or hemorrhage	Chronic Basal ganglia	Subacute stroke	Subacute Large cortical	Subacute Large cortical
Cells used	Ntera-2 cells, the human immortalized tumor cell line		Neural progenitor cells from primordial porcine striatum (xenograft)	Granulocyte-colony-stimulating factor (G-CSF) mobilized CD34+ stem cells	Autologous bone marrow-derived mesenchymal stem cells	Autologous bone marrow mononuclear cells
Cell dose	$2\text{-}6\times10^6$ cells*	5 or 10×10^6 cells*	2×10^7 cells*	15-fold increase with the highest dose of G-CSF	1×10^8 cells*	3×10^8 cells
Manipulation	Neuronal differentiated with retinoic acid†		Fetal porcine striatum was washed, triturated, and dissociated to yield cell suspensions†	Subcutaneous injection of human recombinant G-CSF	Ex vivo culture-expansion using fetal bovine serum†	Isolation using 5% human serum albumin-containing media
Mode of application	Intra-lesional‡		Intra-lesional	Subcutaneous	Intra-venous	Intra-arterial§
Presumed mechanisms	Cell replacement		Cell replacement >trophic support	Trophic support >replacement	Trophic support >replacement	Not specified
Adverse effect	None	1 seizure, 1 syncope, 1 subdural hematoma	1 seizure, 1 worsening of weakness	No difference between the groups	None	None
Preclinical safety test	Not specifically mentioned		Cell viability PCR testing for porcine endogenous retrovirus	Not specifically mentioned	Cell viability Mesenchymal stem cell surface markers Bacteria, fungi, viral and mycoplasma culture	Bacteria and fungi culture

*Equivalent to preclinical studies. †'More than minimal manipulation' by the food and drug administration (FDA) regulation on cell therapy. ‡Immunosuppressed after surgery. §Infusion slowly with transcranial doppler and electroencephalogram monitoring.

determined. The appropriate cell type, treatment mode, and application time will depend on the target mechanism required (i.e., cell replacement *vs.* trophic support) (54). On the other hand, target mechanisms will depend on the characteristics of stroke patients (location and chronicity of lesions). Thus, more detailed guidelines stratified by mechanisms of action (cell replacement *vs.* trophic supports) are needed to obtain maximal benefits in patients with different situations after stroke. Because of the experimental nature of the treatment, clinical trials of cell therapies for stroke have been performed in severely-disabled patients or chronic stroke patients (even several years after stroke onset). However, it may be difficult to demonstrate therapeutic benefit in these patients. Stimulation of stroke-induced neurogenesis, which occurs in certain areas, including the subventricular region, is an important mechanism of MSC therapy (Fig. 2). Unfortunately, patients with severe stroke often have severe damage to the peri-ventricular areas, resulting in limited/damaged endogenous neurogenesis (Fig. 2). Additionally, these patients are often unable to participate in active rehabilitation. We have recently demonstrated that patients who received intravenous autologous MSCs but had extensive subventricular lesions (suggesting damaged neurogenesis system) showed a worse prognosis when compared to those with intact regions of neurogenesis system (Internation Stroke Conference, 2009 Feb, San Diego). Persistent occlusion at the time of intravenous administration of ex vivo culture-expanded MCSs may limit the migration of cells to the injured area of the brain. These patients may not be good candidates for cell therapy for trophic support and neurogenesis stimulation but they may still be candidates for cell replacement therapy. Lastly, tissue levels of Stromal cell-derived factor-1 (SDF-1, also known as CXCL12, a chemokine) may be variable among patients with ischemic stroke. The same thing may be true for cell therapy for cellular replacement strategy. The beneficial effects of intra-lesion application of cells may be limited at the chronic stage; migration and functional integration of transplanted cells with nearby neurons may be limited by scarring (gliosis) and Wallerian degeneration.

Last but not least, pre-treatment screening for safety is mandatory and should be performed extensively. Unlike pharmaceutical products, many stem cell-based products may originate in academic laboratories where researchers are unfamiliar with the applicable regulations (55). FDA regulations for stem cell-based therapy are available (55). Beside the screening tests for viability and other microbiology tests on the cells, patients received tests, including PCR testing for porcine endogenous retrovirus (47), flow cytometry to measure the expression of stem cell surface markers (49), and transcranial Doppler and electroencephalogram monitoring during intra-arterial infusion of bone marrow mononuclear cells (50). We recently reported that intra-arterial infusion of autologous MSCs causes small spotty lesions on diffusion-weighted imaging, suggesting microembolism, although none of the patients showed neurological deterioration (56). In addition, penicillin-sensitivity tests and MSC skin test (subcutaneous injection of small amount of MSCs to test immune sensitization)

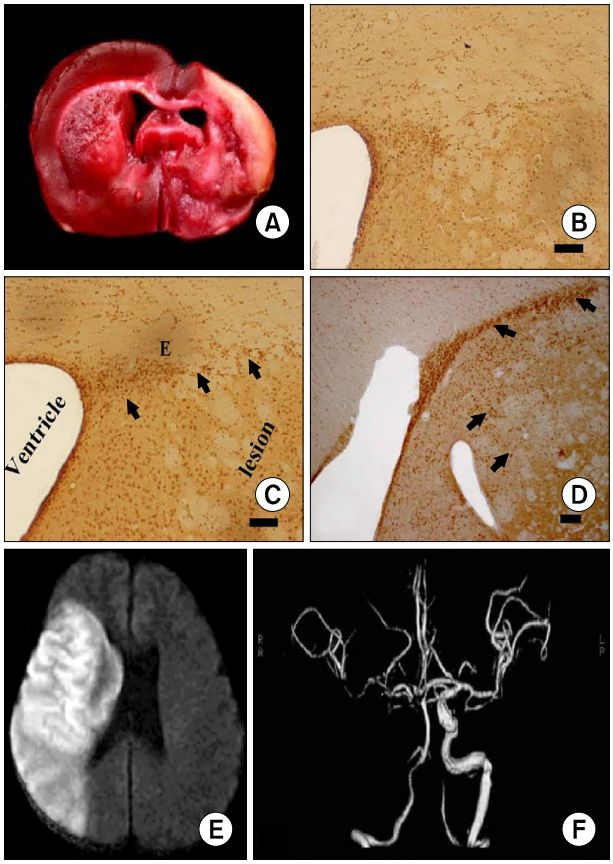

Fig. 2. Discrepancy between pre-clinical and clinical trials (modified from Li, et al. (9) and Bang, et al. (49)). Stimulated neurogenesis after application of human MSCs in transient ischemic rat model (upper lane). (A) 2, 3, 5-triphenyltetrazolium chloride (TTC) staining showing ischemic lesions 14 days after 2-h transient middle cerebral artery occlusion. Bromodeoxyuridine (BrdU) immunostaining in the subventricular zone of the ipsilateral hemisphere at day 14 showed enhancement of neurogenesis in the treated group (stroke rats that received intravenous human MSCs) (D) compared to the sham operated rat (B) and placebo-treated stroke rat (C). One example of autologous hMSC application in stroke patients (lower lane). DWI showed massive infractions involving most of the subventricular area (E). MR angiography showing persistent occlusion at the time of intravenous administration of autologous MSCs (F).

were checked before patients received intravenous autologous MSCs (49). When stem cell-based products involve more than minimal manipulation (such as expansion or differentiation), the cells will probably be grown in culture; this process could involve the use of non-human serum. The most problematic, unresolved issue is the risk of prion transmission and stimulation of immunogenicity by the use of fetal calf serum (FCS) or fetal bovine serum (FBS) in cell culture. These are the most widely used growth supplements for cell culture, and most clinical trials have employed human MSCs expanded in FCS or FBS under FDA-approved protocols. However, FCS may contain potentially harmful xenogeneic components. A single injection of 100×10^6 human MSCs grown under standard conditions (20% FCS) would carry with it approximately 7 to 30 mg of calf serum protein (57). There have been increasing efforts to avoid these risks by using autologous serum or serum-free medium (58). Research in this area should accompany stroke stem cell research until a satisfactory solution is found.

Dr. Wechsler and the Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS) committee suggested guidelines for both pre-clinical and clinical trials on stem cell therapies in stroke (54). The Stroke Therapy Academic Industry Roundtable (STAIR) recommendations were developed to improve the quality of pre-clinical and clinical research on neuroprotective drugs and to reduce the gaps between them (59). As in the neuroprotective field, bench-to-bedside gaps exist as mentioned above. Like the STAIR recommendation, the STEPS recommendations will provide important steps helping steer pre-clinical and clinical research of stem cell therapies and reducing the bench-to-bedside gaps. Unlike with neuroprotective agents, the mechanisms of action of stem cell therapies are still unknown. Hopefully, with better understanding of cell therapy mechanisms of actions, the next STEPS recommendations will provide more detailed guidelines stratified by action mechanisms.

Table 3. Ongoing studies in stem cell research for ischemic stroke

Category	Purpose	References
1. Enhancement of therapeutic efficacy		
Endovascular mode of application	To avoid first passing effect and increase stem cell Numbers in target regions	(56, 67, 68)
Blood-brain barrier manipulation	To increase lesion trophic factor levels or stem cell numbers	(60)
Ex vivo treatment to enhance transdifferentation capacity of stem cells	Neurogenin or trophic factors	(69)
Genetically modified stem cells	Bdnf gene-modified msc	(34, 63)
	Trophic factors other than bdnf	(36)
Stem cell homing and trophism	Enhance chemokine - nitric oxide donor	(70, 71)
Ischemic preconditioning	1. Stem cell protective strategies	(61)
	2. Enhancing trophic supports (auto- and paracrine effects)	(62)
	3. Enhancing proliferation and differentiation of mscs	
2. Feasibility and easy to obtain cells		
Expansion of MSCs with maximized condition	To get larger amount of stem cells within relatively short period Maintain multi-potency and capacity of trophic support	(9, 72)
Various sources of adult stem cells	Adipose stem cells	(73)
	Hematopoietic stem cells	(74)
	Allogenic mscs in non-cns disease	(75-78)
3. Selection of patients		
Patients' characteristics (Age and severity of neurologic deficits)	To select optimal patients for cell therapy	(52, 53)
Characteristics of stroke (location, time after stroke, permeability of blood-brain barrier, and blood flow to infracted tissue)		
Levels of chemokines in either serum or CSF		
4. Safety profiles		
Protocol for toxic screening	To prevent transmitting disease or contamination	(55)
Serum free media	To avoid the use of xenogeneic serum (zoonoses)	
Autologous serum	To avoid the use of xenogeneic serum (zoonoses)	(57, 66, 79)

Perspectives

The great potential for improving therapeutic efficacy and safety of stem cell approaches requires further preclinical and clinical trials (Table 3). Efforts to enhance the therapeutic effects of MSCs (including blood-brain barrier manipulation (60), ischemic pre-conditioning (61, 62), and genetically modified MSCs (34, 36, 63), and to reduce potential adverse effects (use of culture media without xenogeneic serum (58, 64-66) may improve therapeutic outcomes with MSCs.

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Potential Conflict of Interest

The author has no conflicting financial interest.

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