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Stem cell therapy for acute cerebral injury: What do we know and what will the future bring?

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Abstract

Purpose of review—The central nervous system has limited capacity for regeneration after acute and chronic injury. An attractive approach to stimulate neural plasticity in the brain is to transplant stem cells in order to restore function. Here we discuss potential mechanisms of action, current knowledge and future perspectives of clinical stem cell research for stroke and traumatic brain injury.

Recent findings—Preclinical data using various models suggest stem cell therapy to be a promising therapeutic avenue. Progress has been made in elucidating the mechanism of action of various cell types used, shifting the hypothesis from neural replacement to enhancing endogenous repair processes. Translation of these findings in clinical trials is currently being pursued with emphasis on both safety as well as efficacy.

Summary—Clinical trials are currently recruiting patients in phase I and II trials to gain more insight in the therapeutic potential of stem cells in acute cerebral injury. A close interplay between results of these clinical trials and more extensive basic research is essential for future trial design: Choosing the optimal transplantation strategy and selecting the right patients.

Keywords

Neural repair; recovery; stem cell therapy; stroke; traumatic brain injury

Introduction

Stroke causes 1 in 18 deaths in the US and stroke incidence is expected to increase over the next decades.[1] The only effective treatment, intravenous tissue plasminogen activator, has a narrow time window, thereby limiting its application to a small subset of patients with ischemic stroke.[2,3] Unfortunately, many patients are disabled after stroke and require

ongoing medical care, so that most yearly costs occur in this chronic setting.[4] These findings have led to research focused on mechanisms of neural repair as they relate to patterns of human recovery after stroke.

Traumatic brain injury (TBI) is a major cause of mortality and morbidity, especially among young adults, and lifelong disability is common in those who survive.[5] With no effective therapy available, interest in the potential of stem cell therapy for TBI patients is emerging. Stem-cell-based approaches therefore could provide new therapeutic avenues to restore neurological function in both patients with stroke[6] and TBI[7].

Preclinical studies using human cells

A large variety of human cell types have been used in experimental stroke (reviewed in Bliss et al.[8]) and can be divided in neural stem cells (NSCs) and non-NSCs approaches.

NSCs can give rise to neurons, astrocytes or oligodendrocytes. It is unclear which would be the most favorable cell type and how NSCs would be best prepared and modified in order to differentiate into a specific phenotype. NSCs can be generated from human embryonic and fetal stem cells. Using different techniques, NSCs have improved functional recovery after transplantation in experimental stroke.[9-12] The major concern of these cells is their capacity to form tumors, although tumorigenicity is lower for fetal-versus embryonic-derived NSCs.[13] Another approach might be to use induced pluripotent stem cells (iPSCs) generated from reprogrammed somatic cells, such as fibroblasts, to generate NSCs.[14]

Expandable neuroepithelial-like stem cells generated from human iPSCs have also been shown to improve functional recovery.[15] A potential advantage of this technology is that patients' own cells can be used, thereby reducing the risk of cell rejection; the danger of tumor formation, however, remains.

With clinical translation of NSC transplantation underway, it is important to understand the mechanism of action for improved recovery. The initial hypothesis was that NSCs would replace lost neurons and circuits. Indeed successful synaptic integration has been documented, which could contribute to behavioral improvement.[12,16,17] However, evidence for widespread afferent and efferent neuronal projections is lacking. Transplantation of cortical neurons could be a future therapeutic option to improve integration and functioning of transplanted cells.[18] Introducing bioengineered platforms at the time of transplantation could also promote replacement of lost connections and circuitry. [19] Other modes of action have been explored with documented beneficial effects on immunomodulation, neovascularization, neural plasticity and endogenous neurogenesis. [9,20-24]

Various other human non-NSCs transplantations have improved functional recovery mostly with blood, umbilical cord and bone-marrow-derived (non-hematopoietic) cells, mesenchymal stem cells[25-32] and multipotent adult progenitor cells[33]; and in fewer studies with human embryonic[34], placental[35] and amniotic-derived[36] mesenchymal stem cells. These cells are of clinical interest as they—particularly bone marrow-derived cells—are easy to obtain from autologous donors without immunogenic and tumorigenic

complications. There is no convincing evidence that these bone-marrow-derived cells functionally integrate into the brain, but various other mechanisms of action that improve endogenous repair mechanisms have been described, including secretion of neurotrophic factors[28] as well as promotion of neurogenesis[26,30], angiogenesis[27,31] and axonal plasticity[25]. Transplantation of human umbilical cord blood cells by systemic delivery has led to functional improvement by enhancing endogenous repair processes[29,32] and immortalized neural cell lines have been shown to improve functional recovery[37,38]; tumorigenicity, however, remains an issue.

Cell transplantation has also been examined in animal models of TBI using similar approaches to experimental stroke. The pathophysiology of TBI is still not clearly understood, however, thus complicating the search for optimal stem cell therapies. One can hypothesize that replacement strategies should be aimed at transplanting cells that can differentiate into endothelial cells, neurons, glia and oligodendrocytes—for remyelination, especially when considering diffuse axonal injury. Human fetal- and embryonic-derived NSCs have been shown to improve motor and cognitive function by producing neurotrophic factors, increasing angiogenesis and reducing astrogliosis.[39-44] Human blood, umbilical cord, placental and bone marrow progenitor, and mesenchymal stem cell transplantation in animal models of TBI has improved behavioral outcome. The production of neurotrophic factors could underlie this functional amelioration.[40,45-49] Limited evidence of a role for immortalized neural cell lines has been shown by transplanting NT2N human neuronal cells to improve cognitive outcome.[50]

From preclinical research to translation

Translation of preclinical data to human clinical trials is confronted with many difficulties. Experimental studies that have used a wide variety of cells lack direct large scale comparisons to help determine which cells are optimal to use in humans. Use of autologous bone marrow and potentially umbilical cord cells eliminates the risk for immune rejection. When autologous bone marrow cells are harvested by simple sorting on a Ficoll column they can be used in the subacute or chronic phase (> 24 hours) after stroke and TBI. Allogeneic bone marrow and umbilical cord stem cells can be ready for infusion or transplantation earlier after the cerebral event as well as in the subacute and chronic phase, but risk of rejection is a concern. Immunosuppression regimens may therefore be necessary, at least for some period of time. Use of NSCs might be promising, considering their potential for neural replacement, but the risk of teratoma formation must be addressed and closely monitored, and rejection of embryonic- or fetal-derived NSCs even with immunosuppression remains a possibility. Future strategies using allogeneic iPSCs will face similar hurdles and the risk of rejection can only be dealt with by using *autologous* iPSCs. This latter technology, however, is not available in the acute setting because the time necessary for induction and differentiation of these cells exceeds the time frame of acute stroke and TBI (hours to day).

After establishing which cell type to use, the dose, timing and route of administration must be determined. Larger cell numbers are associated with smaller infarct sizes[29,51], but the association with specific processes of neural repair is less clear and there is no established optimal cell dosage. Using higher cell concentrations and numbers will not necessarily result

in increased efficacy. Optimal transplant timing depends on several factors, including preclinical results, cell type, hypothesized mechanism of action and route of administration. Most experimental data have been generated from transplantation within 24 hours after stroke onset, but cell therapy initiated up to 4 weeks after stroke[32] has been shown to significantly enhance functional recovery. A strong association between functional efficacy and transplant timing after stroke could not be determined, but there was an association between structural outcome and time of intervention.[52] Interestingly, stroke and TBI might have different optimal timing patterns. Studies in experimental stroke suggest there is greater functional recovery from mesenchymal stem cell and NSC transplantation at 24 hours compared to 7 days after stroke[53,54], with opposite results in experimental TBI[55].

The proposed mechanism of action of cells used might influence the timing of transplantation. If a neuroprotective effect of the therapeutic intervention is suspected, acute delivery (within 24 hours) may be essential. In contrast, endogenous repair mechanisms exert their greatest potential in the subacute phase after stroke (within the first month in rodents and within 3 months in humans)[56], thus this timing might be most optimal to promote endogenous functional recovery. Hyperacute administration, however, might be less beneficial due to the hostile environment during this phase.

The mode of delivery is also influenced by various factors such as type of cell used, timing, as well as safety and efficacy profiles. Intracerebral (IC) and intracerebroventricular (ICV) techniques precisely administer cells to a chosen location, but may have higher risks due to their invasive procedures. Homing of cells in the brain has been shown to be highest with IC administration compared to intra-arterial (IA) and intravenous (IV) delivery,[57] but whether this correlates with functional improvement has not been established. Systemic delivery might be safer and more feasible, particularly in the acute and subacute phase after stroke onset. One study found that IA infusion compared to systemic IV delivery increased homing around the ischemic lesion, but with no difference in therapeutic benefit[58]. An analogous difference in engrafting after IA versus IV delivery has been described in TBI[40]. Invasive intraparenchymal delivery of cells may not be required for efficiency when neural replacement is not expected—although there may still be a therapeutic advantage with the local release of cell-secreted paracrine molecules and proteins in the peri-infarct region—and systemic infusion may be the preferred mode of delivery for these transplantation strategies from a safety perspective.

Current clinical experience

The first few clinical trials in stroke patients used invasive techniques followed then by studies that mainly applied intravenous infusion delivery strategies (Table 1). IC transplantation of cultured neuronal cells derived from a teratocarcinoma cell line was safe in all 26 patients studied.[59,60] The primary endpoint was the same between the active versus control group, but several secondary functional outcome measurements showed improvement compared to controls.[60,75] After IC transplantation of porcine fetal cells in 5 patients this phase I trial was stopped due to serious adverse events in 2 patients.[61] The first experience with human cells was obtained by transplanting a cell suspension from immature nervous and hemopoietic tissues into the subarachnoid space in 10 patients.

During a 6-month follow-up a potential functional benefit in contrast to a clinically comparable control group was demonstrated.[62] All other recent studies have used autologous bone-marrow-derived cells. One trial showed IC administration of bone marrow mononuclear cells to be safe.[63] The other 8 trials used either IA or IV infusion for delivery of autologous bone-marrow-derived cells (bone marrow mononuclear cells in 6; and mesenchymal stem cells in 3) and demonstrated safety and feasibility.[64-73] IA infusion, used in 3 studies, did not result in functional improvement. The single trial evaluating both mononuclear and mesenchymal stem cells could only demonstrate a functional benefit with mononuclear cells [71]. This potential was underscored by the largest phase II trial in 52 patients with this cell type [66]. Interestingly, very limited preclinical data exists on the use of human bone marrow mononuclear cells[76] although this was the most commonly used approach in published trials. Forty-six percent (6/13) of all studies included patients within the 3-month period following stroke in which endogenous repair mechanisms are known to exert their greatest potential.

The first clinical trial of 97 TBI patients in whom mesenchymal stem cells were intrathecally injected was recently published. Results suggest that this stem cell therapy is safe and potentially efficacious, with documented improvement in motor functions of patients with motor disorders and in consciousness of persistent vegetative state patients, compared to baseline.[74]

Future trial perspectives

There is still insufficient evidence that stem cell therapy is beneficial for acute cerebral injury. Regarding current and future clinical studies, 29 trials registered for stroke patients are: completed (4); ongoing, not recruiting (3); recruiting (17); or not yet recruiting (5) (Table 2). In TBI only 5 trials have been designed (Table 2). In these current trials most of the inclusion criteria for stroke patients require shorter therapeutic time windows compared to the published trials, although exclusive chronic patients are still being recruited in 28% (8/29). IV delivery of autologous bone-marrow-derived mononuclear cells or mesenchymal stem cells seems to be the most preferred strategy, likely because these cells are easy to obtain, simple to deliver, safe, and there is no need for immunosuppressants. An ongoing trial using IC NSC delivery has promising preliminary 6 month and 1 year outcomes[77]. Primary outcome endpoints include feasibility, safety and tolerance, along with functional improvement using the National Institutes of Health Stroke Scale Score (NIHSS), the modified Rankin Scale (mRS) and the Barthel Index. Potentially more extensive scales are needed for functional improvement to be demonstrated [78].

Although the beneficial effects of mesenchymal stem cells have been demonstrated in various experimental designs, as mentioned earlier, the mechanism of action of human bone-marrow-derived mononuclear cells in animal models remains uncertain. Future preclinical data will hopefully provide answers about optimal timing, route of administration and cell numbers. Ideally the most successful experimental design from rigorous preclinical studies should be translated to human clinical trials, although generalizing from animal models to humans is always difficult. Many transplantation strategies use a heterogeneous population of cells and which subtype in this heterogeneous population is of importance for the

therapeutic benefit has to be clarified. Initially neuronal replacement by NSCs was thought to be the mechanism of action for stroke recovery. More recently it is thought to involve enhancement of endogenous plasticity by modulation of inflammation, promotion of angiogenesis, increased native axonal and dendritic outgrowth, neurotrophic effects and neuroprotection[79]. However, integration of transplanted cells in the brain needs further study in order to fully explore the potential of neuronal replacement. Characterization of transplanted cell differentiation in relation to functional improvement is required to determine which cells and cell subtypes are therapeutically effective and to understand the processes of integration into existing neural circuitries.

Elucidating mechanisms of action is important for selection of both cells and patients. Considering the relatively small sample sizes in the currently enrolling clinical trials it is unlikely that the full therapeutic potential of stem cells in stroke and TBI will be revealed in the near future. Trial results will hopefully provide insight into patient selection in relation to cell type used. One can hypothesize that lesion location and size might determine the optimal approach; for example functional recovery in smaller lesions would not require replacement strategies with higher procedural risks, but might benefit from techniques that increase endogenous repair mechanisms. Noninvasive imaging of transplanted cells will be essential to evaluate their survival, distribution, function and effects in patients.[80] Novel findings in experimental models and clinical trials should be combined for optimal phase III trial designs.

Conclusion

Several stem cell therapies tested in animal models for acute cerebral injury have provided preclinical evidence of improved functional recovery. The mechanism of action for many of these strategies, however, is not yet completely elucidated and clinical translation requires additional time. A ‘one size fits all’ therapy is unlikely, considering the heterogeneity of the cells used and the clinical diseases (both stroke and TBI encompass a wide range of acute cerebral insults). However, we have gained significant knowledge from preclinical studies about the mechanisms underlying recovery of neurologic function, and from early clinical trials about the safety and feasibility of cellular therapy for acute cerebral injury. The next decade of stem cell research in stroke and TBI will need to focus on additional basic and clinical research to fully explore the potential of this therapeutic avenue: From bench-to-bedside and just as importantly, from bedside-to-bench.

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Key points

- Preclinical research of cell-based approaches after acute cerebral injury to ameliorate recovery is promising
- Future basic research strategies should be aimed at identifying optimal cell type and numbers to use as well as comparing optimal routes and timing of delivery
- Elucidating mechanisms of action of various stem cells in brain repair is crucial for the further development of cell-based therapies in stroke
- Clinical translation is emerging, which is focused on safety, feasibility and functional improvement
- Ongoing feedback between clinicians and experimental researchers is necessary to improve future clinical phase III trial design

Table 1

Published pilot clinical trials in stroke and TBI

	Study Phase (Author, year)	Cell type	Route of administration	Time after stroke	Sample size	Results
STROKE						
1	I (Kondziolka, 2000) [59]	Cultured neuronal cells derived from a teratocarcinoma cell line	IC	7-55 months	12	Neuronal transplantation is safe and feasible
2	II (Kondziolka, 2005) [60]	Cultured neuronal cells derived from a teratocarcinoma cell line	IC	1-6 years	14 + 4 controls	Functional improvement was documented, but no difference in the primary outcome measurement
3	I (Savitz 2005) [61]	Porcine fetal cells treated with an anti-MHC class I antibody	IC	1.5 – 10 years	5	Cortical vein occlusion and seizures; study stopped after 5 patients
4	I (Rabinovich, 2005) [62]	Cell suspension from immature nervous and hemopoietic tissues	ICV	4-24 months	10	No serious complications; potential functional benefit
5	I (Suárez-Montesagudo, 2009) [63]	Autologous bone marrow mononuclear cells	IC	1-10 years	5	Safe
6	I (Barbosa da Fonseca, 2010) [64]	Autologous bone marrow mononuclear cells	IA	59 - 82 days	6	Safe and feasible
7	I/II (Bang, 2005) [65]	Autologous human mesenchymal stem cells	IV	< 7 days	5 + 25 controls	Safe and feasible profile with potential functional benefit
	I/II (Lee, 2010) [66]	Autologous human mesenchymal stem cells	IV	< 7 days	16 + 36 controls	Safe and feasible profile with potential functional benefit
8	I (Battistella, 2011) [67]	Autologous bone marrow mononuclear cells	IA	< 90 days	6	Safe and feasible
9	I (Savitz, 2011) [68]	Autologous bone marrow mononuclear cells	IV	24-72 hours	10	Safe and feasible
10	I (Homou, 2011) [69]	Autologous bone marrow human mesenchymal stem cells	IV	36–133 days	12	Safe and feasible
11	I/II (Bhasin, 2011) [70]	Autologous bone marrow human mesenchymal stem cells	IV	7-12 months	6 + 6 controls	Safe and feasible
	I/II (Bhasin, 2012) [71]	Autologous bone marrow mononuclear and mesenchymal stem cells	IV	3 months – 2 years	20 + 20 controls	Safe and improvement of modified Barthel Index

Study Phase (Author, year)	Cell type	Route of administration	Time after stroke	Sample size	Results
12 I (Prasad, 2012) [72]	Autologous bone marrow mononuclear cells	IV	7-30 days	11	Safe and feasible
13 I/II (Moniche, 2012) [73]	Autologous bone marrow mononuclear cell	IA	5-9 days	10 + 10 controls	Safe and feasible; no clear functional improvement

TBI

1 I (Tian, 2013) [74]	Autologous bone marrow mesenchymal stem cell	IT	> 1 month	97	Safe and feasible; potential benefit of motor function
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IA: Intra-arterial; IC: Intracerebral; ICV: Intracerebroventricular; IT: Intrathecally; IV: Intravenous; TBI: Traumatic brain injury

Table 2

Registered clinical trials in stroke and TBI

ID	Study Phase	Cell Type	Route of administration	Time after stroke	Primary outcome measurement	Region	Recruitment status
STROKE							
NCT00950521	II	Autologous peripheral blood CD34 ⁺ Stem cell	IC	6-60 months	NIHSS	Taiwan	Completed
NCT01518231	I	Autologous hematopoiesis stem cell	IA	<1 year	NIHSS	China	Recruiting
NCT00473057	I	Autologous bone marrow cells	IV/IA	3-90 days	New deficits	Brazil	Completed
NCT01468064	I/II	Autologous bone marrow stromal cell and endothelial progenitor cell	IV	5 weeks	Adverse events	China	Recruiting
NCT00908856	I	Autologous bone marrow mononuclear cell	IV	<72 hours	Death	United States	Not yet recruiting
NCT01028794	I/IIA	Autologous bone marrow mononuclear cells	IV	7-10 days	NIHSS	Japan	Recruiting
NCT01501773	II	Autologous bone marrow mononuclear cells	IV	7-30 days	Barthel index	India	Completed
NCT00859014	I	Autologous bone marrow mononuclear cells	IV	24-72 hours	Safety and feasibility	United States	Ongoing, not recruiting
NCT01832428	I/II	Autologous bone marrow mononuclear cells	IT	Unclear	Improvement in power of muscles	India	Recruiting
NCT01273337	II	Autologous bone marrow ALD-401	IA	13-19 days	Safety (various parameters)	United States	Recruiting
NCT00535197	I/II	Autologous CD34 ⁺ subset bone marrow stem cell	IA	<7 days	Adverse events	United Kingdom	Recruiting
NCT00761982	I/II	Autologous CD34 ⁺ stem cells	IA	5-9 days	Adverse events & new deficits	Spain	Completed
NCT01716481	III	Autologous mesenchymal stem cell	IV	<90 days	mRS	Republic of Korea	Recruiting
NCT00875654	II	Autologous mesenchymal stem cells	IV	<14 days	Feasibility and tolerance	France	Recruiting
NCT01714167	I	Autologous bone marrow mesenchymal stem cell	IC	3-60 months	NIHSS	China	Recruiting
NCT01461720	II	Autologous mesenchymal stem cells	IV	1 week - 2 months	NIHSS, mRS, Barthel index	Malaysia	Recruiting

ID	Study Phase	Cell Type	Route of administration	Time after stroke	Primary outcome measurement	Region	Recruitment status
NCT01091701	I/II	Ex vivo cultured adult allogenic mesenchymal stem cells	IV	< 10 days	Adverse events and NIHSS	Malaysia	Not yet recruiting
NCT01436487	II	Adult stem cell: Multistem	IV	1-2 days	Adverse events and mRS	United States	Recruiting
NCT01849887	I/II	Allogeneic bone marrow mesenchymal stem cells	IV	24-72 hours	Adverse events	United States	Not yet recruiting
NCT01297413	I/II	Allogeneic bone marrow adult mesenchymal stem cells	IV	>6 months	Safety (various parameters)	United States	Recruiting
NCT01287936	I/IIA	Modified stromal cells (SB623)	IC	6-60 months	Safety (various parameters)	United States	Recruiting
NCT01453829	I/II	Autologous adipose-derived stromal Cells	IV/IA	Unclear	NIHSS, Adverse events, Barthel index	Mexico	Recruiting
NCT01678534	II	Allogenic mesenchymal stem cells from adipose tissue	IV	<12 hours	Adverse events, complications and tumour development	Spain	Not yet recruiting
NCT01310114	IIA	Human placenta-derived cells, PDA001	IV	Subacute	Adverse events and mRS	United States	Ongoing, not recruiting
NCT01673932	I	Umbilical cord blood mononuclear cells	IC	6-60 months	NIHSS and safety (various parameters)	China	Recruiting
NCT01438593	I	Umbilical Cord Blood CD34 ⁺ Stem Cell	IV	6-60 months	NIHSS	Taiwan	Not yet recruiting
NCT01700166	I	Autologous human cord blood derived stem cell	IV	Children only: 6 weeks - 6 years	Functional, physiological and anatomic outcome	United states	Recruiting
NCT01151124	I	CTX0E03 neural stem cells	IC	6-months - 5 years	Adverse events	United Kingdom	Ongoing, not recruiting
NCT01327768	I	Olfactory ensheathing cells	IC	6-60 months	NIHSS	Taiwan	Recruiting

TBI

ID	Study Phase	Cell Type	Route of administration	Time after stroke	Primary outcome measurement	Region	Recruitment status
NCT01575470	I/II	Autologous bone marrow mononuclear cells	IV	< 36 hours	Neurological events (incl. CVA)	United States	Recruiting
NCT01451528	?	Allogeneic umbilical cord blood	IV/IA	< 6 months	Short-term memory	Republic of Korea	Not yet recruiting
NCT00254722	I	Autologous bone marrow precursor cell	IV	Children only: < 36 hours	Adverse events	United States	Completed
NCT01251003	I/II	Autologous umbilical cord blood	IV	Children only: 6-18 months	Safety	United States	Recruiting
NCT01851083	I/II	Autologous bone marrow mononuclear cells	IV	Children only: <36 hours	Brain white matter and gray matter structural preservation	United States	Not yet recruiting

IA: Intra-arterial; IC: Intracerebral; IT: Intrathecal; IV: Intravenous