Review

Stem Cell Treatments in Preclinical Relevant Stroke Models

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ABSTRACT: Since stroke has limited treatment options, an active search for new therapeutic approaches is required. Initial excitement of using cell-based therapies to stimulate recovery processes in the ischemic brain turned into a more measured perspective, acknowledging obstacles related to the unfavorable environments associated in part with aging. Given the predominance of stroke in older populations, evaluating the effectiveness of cell therapies in aged brain environments is essential and clinically relevant. Despite a common perception of the aged brain being resistant to regeneration, recent research with neural precursor cells and bone marrow-derived mesenchymal stem cells indicates that cell-based therapy can promote plasticity and remodeling in the aged rat brain. However, significant differences in the aged brain compared to the young brain, such as expedited progression of ischemic injury to brain infarction, decreased rate of endogenous neurogenesis, and delayed onset of neurological recovery, must be noted. The effectiveness of cell-based therapies may further be connected to age-related comorbidities such as diabetes or hyperlipidemia, potentially leading to maladaptive or impaired brain remodeling. These age-related factors need careful consideration in the clinical application of restorative therapies for stroke.

KEYWORDS: *Stroke, cell therapy, comorbidities, aging, neurogenesis.*

A Major Risk Factor for Stroke is Ageing

Regardless of expansive progress in comprehending the progression of the ischemic stroke over the past several decades, there is still no therapy capable of successfully protecting neurovascular units (NVU) and substantially improving the neurological dysfunction of stroke patients [1].

The latest literature reveals equivalence for the terms “premature stroke,” “early-onset stroke,” and “stroke among the young/young stroke”.

This may be because of the lack of a definition for what characterizes a premature stroke.

Furthermore, there is no consistency in defining the specific age ranges that classify individuals as "young" or "juvenile."

The lower age threshold remains consistently set at 18 years, but there is variability in the upper age limits, which typically range from 45 to 55 years of age.

Among these upper limits, 45 and 50 are the most commonly chosen cutoff points.

It is important to notice that these threshold points are below the notable age of 55 beyond which the incidence of strokes increases twofold with each advancing decade.

Additionally, it's worth mentioning that they correspond to the lowest quartile of the entire age distribution for stroke [2].

Older patients exhibited a higher prevalence of comorbidities, were administered tPA (tissue-type plasminogen activator) more frequently, and experienced poorer outcomes, irrespective of whether intravenous tPA or thrombectomy was employed.

Moreover, they were more commonly placed in institutional care upon discharge [3].

Stroke in women can be regarded as a distinct phenomenon because it exhibits several differences when compared to strokes in men.

These distinctions encompass particular aspects in epidemiology, etiology and outcome features, alongside distinct pathophysiological mechanisms. Stroke ranks as the second leading cause of death among women worldwide [4].

Aged Animals with Stroke are Clinically More Relevant

The effects of a stroke become more noticeable as age advances.

Hence, the often-underestimated influence of age in animal models of stroke might have consequences for the quality of data and impede the translation of findings from rodent models to human contexts [5].

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In human ischemic stroke, the middle cerebral artery (MCA) and its branches are the cerebral blood vessels most commonly impacted, contributing to approximately 70% of all infarcts.

As a result, techniques that induce occlusion of the middle cerebral artery are the most similar to human ischemic stroke.

Technically, the MCAo version is less intrusive and eliminates the need for craniectomy so it avoids harming the cranial structures.

The common carotid artery (CCA) is being temporarily ocluded in this method, and there is a suture introduced straight into the internal carotid artery (ICA) and the suture is being advanced until it disrupts the blood flow to the middle cerebral artery (MCA).

Introducing the suture into the transected external carotid artery (ECA) and utilizing the ECA trunk as a route for threading a suture through the ICA is another approach.

A valuable tool for ensuring complete MCAo can be Laser Doppler flowmetry.

Due to this technique it is possible induction of either permanent MCAo or transient ischemia with reperfusion.

The ECA technique is a higher desire for temporary MCAo as it keeps the anatomic integrity which is required for reperfusion.

Since it preserves the anatomic integrity needed for reperfusion, the ECA approach is a preferable option for transient MCAo.

Sixty minutes, 90 minutes, and 120 minutes are the commonly used durations of MCAo with a suture or permanent MCAo in rats; the percentage of success of subarachnoid hemorrhage (SAH) is 12% and of inducing an infarction is 88%-100%.

The percentage of success SAH and after transient MCAo of 60 minutes are similar in both mouse models and rats.

This procedure causes territorial infarction in rats, involving the striatum based on the duration of MCAo.

Choosing the adequate animal stroke model is at least partially the accomplishment key of preclinical stroke research in formulating innovative treatments.

The most used rodent stroke models along with their advantages and disadvantages are studied below (Table 1) [6].

Table 1. The most used rodent stroke models along with their advantages and disadvantages.

	Advantages	Disadvantages
Intraluminal suture MCAo model	Mimics human ischemic stroke	Hyper-/hypothermia
	Exhibits a penumbra	Increased hemorrhage with certain suture types
	Highly reproducible	Not suitable for thrombolysis studies
	Reperfusion highly controllable	
	No craniectomy	
Craniotomy model	High long-term survival rates	High invasiveness and consecutive complications
	Visual confirmation of successful MCAo	Requires a high degree of surgical skill
Photothrombosis model	Enables well-defined localization of an ischemic lesion	Causes early vasogenic edema that is uncharacteristic for human stroke
	Highly reproducible	Not suitable for investigating neuroprotective agents
	Low invasiveness	
Endothelin-1 model	Low invasiveness	Duration of ischemia not controllable
	Induction of ischemic lesion in cortical or subcortical regions	Induction of astrocytosis and axonal sprounting, which may complicate the interpretation of results
	Low mortality	Low reproducibility of infarcts
Embolic stroke model	Mimics most closely the pathogenesis of human stroke	Spontaneous recanalization
	Appropriate for studies of thrombolytic agents	High variability of lesion size

Abbreviation: MCAo, middle cerebral artery occlusion.

Natural Stroke Recovery in Elderly Patients and Animal Models

Studies on neuroprotection using animal models remain valuable.

They offer important perceptions into approaches to lower the severity of strokes as they continue to provide translational potential for enhancing future stroke results.

One reason for the ineffectiveness of treatments focused only on protecting neuronal cells may be partially due to the fact that they don't simultaneously protect the cerebral blood vessels from seecondary damage caused by

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inflammation and the effects of reactive oxygen and nitrogen species.

Eventhough anti-inflammatory techniques have shown success in animal models of stroke, their translation to clinical applications has not met anticipated results [7].

Within the initial weeks or months after the stroke, patients recover some of their previously neurological functions and it is probably attributed to the functional readaptation of the damaged area [8].

There are no effective therapeutic procedures currently available for treating elderly individuals, apart from endovascular clot removal, which can be limited by the accessibility of the surgical site.

Prior research has shown that the brain of a young adult has the capacity to trigger its own repair mechanisms in response to ischemic stroke.

Nevertheless, in aged rodents, ischemia-induced angiogenesis and neurogenesis are diminished since in the subventricular area basal cell proliferation is reduced, and in the dentate subgranular zone there is a number of stroke-generated granule cells lower.

Moreover, ischemic stroke in older mice results in axon damage and demyelination, which is linked to deteriorated neurobehavioral outcomes.

Therefore, in aging animals it is important to explore approachable strategies in order to protect against ischemic stroke [9].

A beneficial impact on the regeneration of nerve cells and the reestablishment of neural connections may be due to rehabilitation training.

It can enhance the brain's nutrient supply, the excitability of the nervous system within the cerebral hemisphere, accelerate the healing of damaged brain regions and elevate activity levels.

All of these factors collectively aid in the restoration of brain functions following a cerebrovascular event [10].

Strategies to Enhance Neurological Function and Tissue Restoration Post-Stroke through Cell Therapy

Stem cells are unique cells capable of both self-renewal and differentiating into various cell types.

The nature of stroke pathophysiology makes it a prime candidate for stem cell intervention. Once a stroke occurs, there isn't a neurodegenerative process that impedes recovery.

There are two important approaches for stroke of stem cell therapies:

- Endogenous strategies, which aim to enhance the longevity, mobilization and generation of inherent neural stem cells
- Exogenous approaches, involving the transplantation of cells from an external source into a patient.

These approaches are promising ways to improve the potential of stem cells in promoting the recovery post-stroke.

The areas of the brain surrounding the infarct, known as the ischemic penumbra, hold the highest potential for recovery after a stroke.

Therefore, many neuroprotective treatments aim to minimize damage in the periinfarct area.

While preclinical studies focusing on individual pathways have shown promises in neuroprotection, the results from clinical trials have been disappointing exploring neuroprotective strategies, so in order to achieve comparable outcomes, disturbing multiple pathways may be necessary in humans [11].

Murine induced pluripotent stem cells (iPSCs) are considered a promising cellular source for transplantation therapy, offering the potential to introduce new neurons into the damaged brain following an infarction.

This is because iPSCs maintain a strong capacity for replication and pluripotency, allowing them to differentiate into various cell types comparable to embryonic stem cells (ESCs) [12].

While some research has demonstrated promising outcomes, there are still several obstacles that need to be overcome in order to make cellular transplantation a viable clinical solution for neurological conditions.

If stem cell treatments by themselves fail to provide robust and enduring clinical advantages, combined therapeutic approaches might be the key.

In order to enhance axonal remodeling, cell transplantations could be complemented with optogenetic or electrical stimulation.

Cell transplantation could be performed in conjunction with growth factors, inhibitors of growth factors, or an artificial extracellular matrix.

Furthermore, autologous cells could be modified before transplant to generate neurotrophic factors or rectify a genetic insufficiency, aiming not to completely replace an existing neural population, but to support its function.

iPSCs are highly promising for stroke therapy due to the absence of ethical problems and reduced risk of graft rejection.

Yet, the response of the aged brain to transplanted cells remains somewhat undefined [13].

Stem and Precursor Cells in Subcortical and Cortical Stroke Neurology

When the infarct occurs in the striatum, a subcortical region known for its inherent activity-dependent plasticity, natural recovery is often noticed.

It seems that neurological recuperation is linked to structural dendritic and synaptic plasticity in the unaffected striatum, as noted in animal models [14].

The level of spontaneous functional recovery was significantly impacted by the stroke location, whether cortical or subcortical in rats [15].

In patients who have experienced subcortical infarction with early motor recovery there is an enhanced spontaneous neuronal activity in structurally impaired cortex [16].

However, no important remodelling of corticospinal tract from the non-ischaemic hemisphere linked to effective recovery in rats with subcortical infarcts has been found [17].

The plasticity of undamaged rubral projections facilitates the natural restoration of function following corticospinal tract lesion in mice.

Overall, these findings suggest that stroke location and the involvement of specific neural pathways can impact the extent of spontaneous recovery following stroke [18].

Transplantation of hiPSCs into the striatum of young-adult animals after middle cerebral artery occlusion (MCAO) demonstrates that hiPSCs derived from umbilical cord blood can be differentiated into dopaminergic neurons and transplanted into hemiparkinsonian rats, resulting in reduced proliferation and tumor-like growth [19].

Magnetic resonance imaging (MRI) track the fate of neural stem cells (NSCs) derived from induced pluripotent stem cells (iPS cells) in traumatic brain injury (TBI) of rats, showing successful migration of NSCs to the injured area [20,21].

In a new study, human iPSCs were directly transplanted into the injured neocortex of older rats and not only they survived but they also differentiated into neurons.

This led to noticeable improvements in functional recovery, as observed in the cylinder test results at both 4 and 7-weeks post-transplantation.

Moreover, these grafted human iPSCs inhibited the activation of microglia/ macrophages in the cortex affected by the stroke, as demonstrated by distinct morphological alterations in these cells in animals that received the cell graft compared to those that received a vehicle injection.

iPSCs can be differentiated into specific cell types and transplanted into the striatum with potential for functional recovery and migration to target areas [22].

Decreased level of immunodensity for inducible nitric oxide synthase (iNOS) and also significantly reduced pro-inflammatory M1 macrophages was demonstrated in rats that received neural precursor cell (NPC) in comparison to the control group, so NPC transplantation diminishes the chronic immune environment in spinal cord damage [23].

Some studies showed that the release of osteopontin is the pathway through which microglia stimulate the expansion of neural precursor cells [24].

Compared to other vertebrate species, the number of microglia in telencephalic proliferative zones is greater in human and nonhuman primate species.

Cortical neurons derived from pluripotent stem cells can become part of the neural circuitry in the adult human cortex.

They establish functional connections with existing neurons.

Additionally, there has been developed a technique able to transform pluripotent stem cells into cranial motor neurons, focused on those motor neuron subtypes impacted by specific neurodegenerative conditions.

It has been demonstrated that human fibroblasts can be reprogrammed into induced motor neurons, which had similar properties with spinal motor neurons.

In a rodent model of spinal cord damage, they had the potential to enhance locomotor recovery [25].

The established procedures are able to effectively produce cortical neurons, sensory neurons, midbrain dopaminergic neurons and spinal motor neurons from pluripotent stem cells [26].

Use of Mesenchymal Stem Cells in Striatal and Neocortical Stroke Therapy

An overview of the use of MSCs in ischemic stroke, emphases their ability to modulate the inflammatory environment, stimulate

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endogenous neurogenesis and angiogenesis and reduce glial scar formation.

These findings support the potential of MSCs as a treatment option for stroke.

The utilization of mesenchymal stem cells as a therapeutic intervention during the later stages of a stroke demonstrates their aid in the recovery and rehabilitation process.

This suggests that MSCs might not just be useful immediately after a stroke, but also in the longer-term recovery process [27].

MSCs have shown the ability to diminish post-stroke inflammation in the brain, primarily through the release of TNF-α induced protein 6 (TSG-6).

Moreover, transplantation of MSCs not only ameliorated neurological function, but also managed to decrease the levels of TNF-α, while elevating IL-10 expression in rats post-cerebral ischemia.

Additional research highlighted that a delayed systemic administration of MSCs enhanced motor recovery [28].

Improved functional recuperation in rats after an ischemic stroke emerged from transplanting MSCs sourced from cranial bone (cMSCs) [29].

Umbilical cord blood-derived cells (UCBCs), similar to bone marrow-derived cell, have potential for cell-based therapies in the context of brain injury and neurodegenerative diseases. UCBCs and their potential to generate neural progenitor cells, such as immune tolerance and engraftment kinetics, makes them an attractive source for hematopoietic stem cell transplantation and a promising option for diseases of the nervous system.

The therapeutic capacity of UCBC with their immunomodulatory effects and potential for treating various diseases makes them a hopeful choice [30,31].

According to some studies, MSCs enhanced angiogenesis in diabetic rats with peripheral artery disease (PAD) [32].

When comparing mesenchymal stem cells (MSCs) from individuals with diabetes to those that were healthy, it was observed that those with diabetes had a unique secretome with increased levels of angiogenic factors [33].

However, it also seems that MSCs derived from diabetic rats showed reduced angiogenic capacity and were ineffective in improving limb ischemia [34].

This emphasizes the harmful impact of diabetes on brain morphology and functions, including decreased neurogenesis, decreased brain volumes, increased stroke incidence and high-fat diet-induced hyperlipidemia which leads to cerebral vascular remodeling and worsens neurological outcomes after brain ischemia [35].

Diabetes, both type I and type II, can alter cerebral structure and function, with hypoglycemia and hyperglycemia being linked to cognitive impairments and dementia [36].

Overall, these studies provide evidence that both diabetes and hyperlipidemia can impact post-ischemic brain remodeling.

Combined Treatment and Co-Transplantation Approaches

So far, single-drug treatments aiming to reduce or prevent brain injury after a stroke haven't been successful.

Since stroke influences numerous mechanisms, as shown in past research, such as regeneration, plasticity and physiology of the central nervous system (CNS) and also age-related changes in the adaptive immune system, so the ineffectiveness of single-drug treatments might not be surprising.

In neurorestorative research for stroke therapy, both bone marrow-derived mesenchymal stem cells (BM MSCs) and hematopoietic stem/progenitor cells (HSPC) are the most commonly utilized cells in both preclinical and clinical studies.

Hence, combining the transplantation of BM MSCs with other cell types could improve microenvironment, optimize graft success, and enhance post-stroke functional rehabilitation.

Our current understanding encompasses: (1) even in older brains affected by stroke, the capacity for neurogenesis remains; (2) the aged brain's environment is receptive to BM MSC transplantation; and (3) recovery is evident in certain behavioral assessments, though not consistently in all.

Nevertheless, in upcoming research, there are still considerable developmental and translational challenges to be deal with such as (1) the comprehension of the distinction of different phenotypes, (2) a main challenge is tumorigenesis, (3) therapies targeting neuroinflammation in neurodegenerative diseases and various damages through stem cell treatment are possible objective to promote and (4) physical rehabilitation can improve the effectiveness of cell therapy.

Using BM MSC treatment in older rodents calls for more detailed research. This includes multiple therapeutic cell doses at different post-stroke intervals and trying combinations with G-CSF or other growth factors/cytokines [37].

Conclusions

As a conclusion, (i) Despite advances in understanding ischemic stroke, effective treatments remain elusive. A specific approach, recognizing age and gender intricacies, is crucial for future research and treatment strategies.

(ii) In human ischemic stroke, the middle cerebral artery (MCA) is primarily affected.

While the MCAo version presents advantages like being less intrusive, its success rate in causing infarction is high, but it also bears a risk of subarachnoid hemorrhage.

(iii) Stem cells offer significant potential in post-stroke recovery through both endogenous and exogenous strategies.

The potential of iPSCs is especially promising, but understanding their interaction with the aged brain remains a crucial next step.

(iv) Mesenchymal stem cells (MSCs) exhibit potential therapeutic efficacy in ischemic stroke treatment, attributed to their anti-inflammatory properties, promotion of endogenous neurogenesis and angiogenesis and ability to reduce glial scar formation. Their applicability extends beyond immediate post-stroke interventions to long-term recovery phases.

(v) The potential of combined cell therapies, particularly leveraging BM MSCs with other cell types, offers a promising avenue for post-stroke rehabilitation (Figure 1).

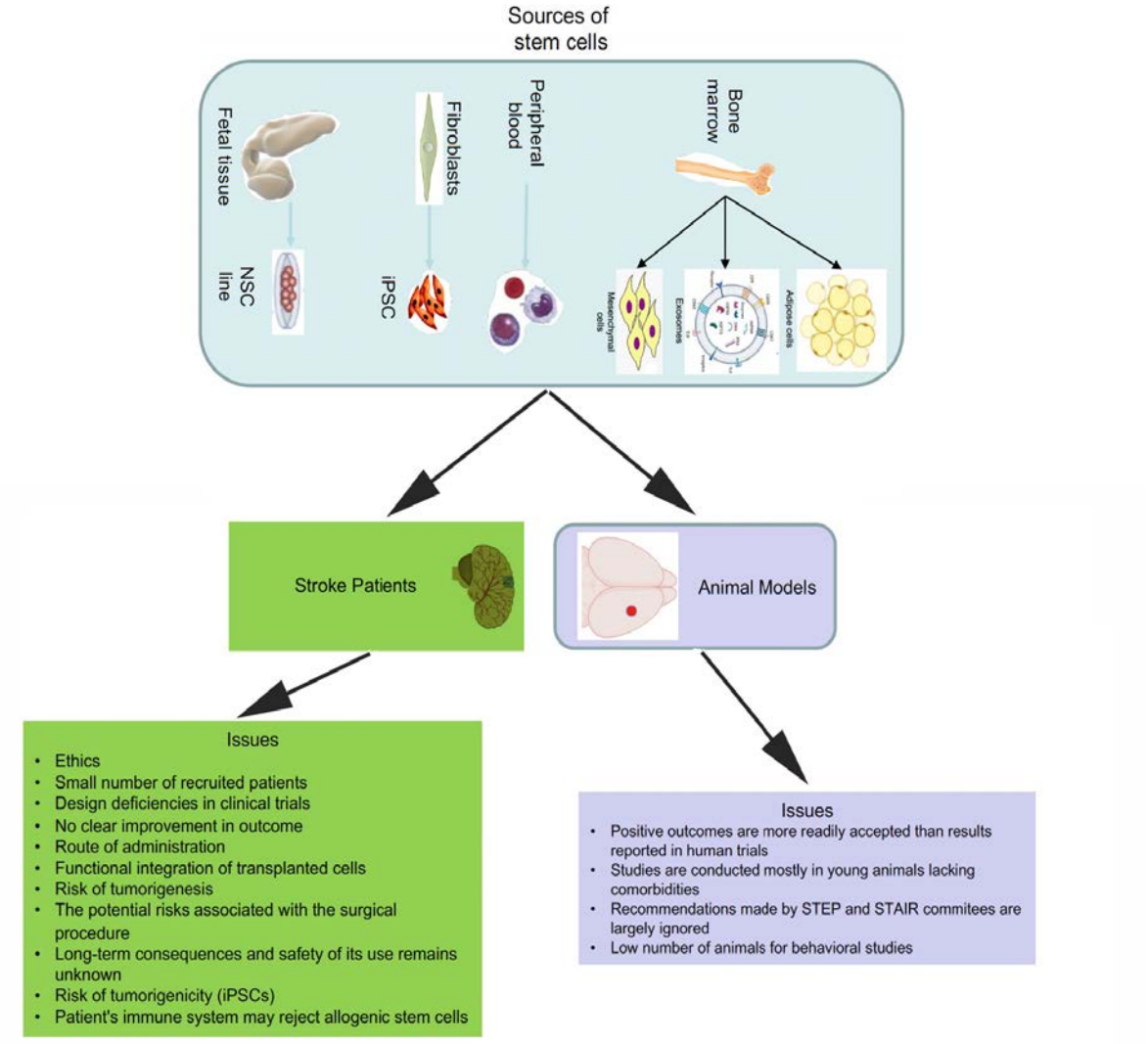

Figure 1. Summary of stem cell treatments in both animal models and humans, emphasizing factors contributing to the challenges in translating animal therapies to humans. Unresolved issues in the realm of stem cell therapy for ischemic stroke encompass ethical considerations, the selection of appropriate cell types, optimal cell dosage, transplantation methods, patient categorization, potential long-term effects and safety concerns, the effective integration of transplanted cells, the risk of tumorigenesis, hazards linked to the surgical procedures, and the likelihood of tumorigenicity, particularly in induced pluripotent stem cells (iPSCs), as well as the potential rejection of allogenic stem cells.

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Conflict of interests

None to declare

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