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Mesenchymal stem cell stroke therapy: current limitations in its clinical translation

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Abstract

For more than a decade now, research studies, proof of concept work, and clinical trials have endeavored to understand how mesenchymal stem cells might be used to help protect, repair, and/or regenerate damaged brain tissue following stroke. To date, the majority of studies have not demonstrated significant improvements in either morbidity or medium-long-term outcome, although safety has been relatively well proven. Limitations are likely to be linked to the pathobiological complexity and seriousness of stroke tissue damage, low efficacy of treatment, and short half-life of bio-active proteins released by stem cells. This article will highlight the heterogeneity and limitation of completed studies and the current status of ongoing work. At the same time, the potential of other combinational type treatments, such as drug-loading and targeting, and the use of hydrogels is discussed.

Keywords

Mesenchymal stem cells, stroke, drug-loading, drug targeting

Introduction

Various *in vivo* stroke models have been utilized even going as far back as the early 2000s, particularly in rodent models of ischemic stroke and these initially showed significant promise. For example, Kang et al. [1], using a rat model of middle cerebral artery (MCA) occlusion (MCAO), showed significant improvement in motor function following lateral ventricle transplantation of adipose-derived mesenchymal stem cells (MSCs) after their differentiation into neural-like cells. In 2010, Leu et al. [2] used a similar model of stroke and administered 2 million adipose-derived MSCs intravenously and found that, over a period of 3 weeks, the infarction size in the test group was notably smaller and, in addition, motor function improved, and markers

of cell apoptosis and inflammation were reduced. Since then, many others have repeated these promising studies, with no less than 53 articles appearing in the PubMed database in 2022 alone.

The first human clinical trial result is identified as far back as 2005 when Bang et al. [3] performed a randomized controlled early phase II study during which 5 individuals who had suffered severe MCAO were given 100 million MSCs delivered intravenously approximately 5 weeks after the infarction. Monitoring over the forthcoming year showed improved outcomes measured using the Barthel and Rankin stroke scales with no serious side effects, thus presenting some reasonably compelling early evidence of potential therapeutic benefit. In this article, the most recent clinical studies will be analyzed and shortcomings and limitations to date described.

Human clinical trial results to date

Systemic delivery

To date, still, the largest phase II clinical trial data comes from the MASTERS-1 Athersys Multistem study, which was a randomized double-blind, placebo-controlled investigation of i.v. delivery of either 400 million or 1.2 billion allogeneic bone marrow (BM)-derived stem cells within 48 h of the initial infarction in 33 centers from UK and USA [4]. All patients had received successful recombinant tissue plasminogen activator (tPA) therapy for recanalization.

There was no significant difference in stroke outcome after 12 months; however, those patients who were initially treated within 36 h, particularly, did show some benefits compared to the placebo group, and a MASTERS-2 phase III clinical trial is currently ongoing (over 200 patients treated to date; no outcome data) [4]. The concept of this type of i.v. treatment is clearly not one of direct involvement of the stem cell mixture in cell replacement, but of the paracrine effects and combination of potentially anti-inflammatory and neuroregeneration-promoting growth factors, and cytokines known to be released from this type of cell that would offer protection, improve blood flow and angiogenesis, and support regeneration of peri-infarcted neural connectivity and plasticity [5].

A similar ongoing but much smaller trial has been listed by Suda et al. [6], originating and the first of its kind in Japan. In this case, dental pulp stem cells are cultured *in vitro* and delivered as an allogeneic therapy i.v. at a dosage of either 100 million or 300 million within 48 h of ischemic stroke [National Institutes of Health Stroke Scale (NIHSS) from 5–20 at baseline]. No resulting outcome data is available yet to assess.

Other completed i.v. delivered trials have been mostly early phase II studies. For example, Law et al. [7] delivered BM-derived autologous MSCs 2 million/kg body weight to 9 patients who suffered severe MCA and had NIHSS scores of between 10–35, within 2 months of the primary infarction. No difference in neurological recovery or functional outcome was seen compared with the placebo control group. Similarly, in the STARTING-2 trial, 39 individuals with severe MCA were treated with autologous MSCs (obtained from the BM) within 3 months of symptoms onset and no difference in recovery was seen at the 3-month end-point between treated and control groups, although neuroimaging revealed some protection against corticospinal tract degeneration possibly supporting motor function recovery [8, 9].

Most recently, de Celis-Ruiz et al. [10] completed the AMASCIS phase II trial with only 4 patients receiving adipose-derived MSCs 1 million/kg body weight i.v. within 2 weeks of tPA recanalized moderate-severe ischemic stroke (NIHSS 8–20), and showed no improvement compared with placebo group over 24 months.

It is important to note the differences in each of the trials related to numbers of cells delivered, timing of the delivery, severity of the initial stroke, and successful tPA recanalization (or not) amongst other parameters, making them very difficult to compare or correlate. A major limitation may of course be the ability of the MSCs to penetrate the blood-brain barrier (BBB) and exert a concerted targeted paracrine delivery effect over time at the infarcted region of the brain. Yarygin et al. [11] describe the function of the BBB and the consequences of its disruption including neurovascular unit disruption, oedema, and inflammation following ischemic stroke. Evidence is summarized by the inability of stem cells to pass even the disrupted barrier *in vivo* after i.v. or i.a. injection although some cells are able to temporarily adhere and remain within cerebral capillaries for up to 72 h [11]. Along the same theme, Bang et al. [12] measured circulating extracellular

vesicle (EV) expression up to 3 months following i.v. injection of autologous BM-derived MSCs in 39 patients (*versus* placebo control group) following ischemic stroke (NIHSS 6–21), in the STARTING-2 trial. Their hypothesis that the EV carries the essential medicinal growth factors and cytokines that may help recovery was partially proven as they showed that circulating EV numbers correlated with improvement in motor function as measured by diffusion tensor imaging and magnetic resonance imaging [MRI; resting state functional MRI (rs-fMRI)]; however, whilst micro-RNAs also increased, trophic factor levels did not. EVs, including those secreted by MSCs, can pass freely through the BBB and hence, are candidates for a targeted drug delivery approach after stroke, but mechanisms to enable continued secretion and focused delivery of their anti-inflammatory and pro-regenerative cargo are yet to be found [13].

Direct *in loco* injection

A more targeted delivery approach involves direct injection or transplant of MSCs into the stroked region of the brain, and this has been attempted primarily in chronic stroke patients in several recent clinical studies [14]. Twenty million human neural stem cells were implanted intra-cerebrally (putamen ipsilaterally to the infarct) by stereotaxic injection into 23 stroke patients between 2–13 months after the original infarct (PISCES-2) [14]. Here, improvements in upper limb function were seen, as measured using the action research arm test (ARAT), remaining to the 12-month end-point of assessment, although, only in those individuals displaying residual function at the start of the study, and of course, a study limitation here is the lack of a placebo or non-treated control group. The first human study was carried out on perinatal arterial ischemic stroke (PAIS) in the PASSIoN open-label intervention study [15]. In this study, approximately 50 million BM-derived MSCs were administered intra-nasally in 10 neonates with PAIS. Without adverse events (AEs), whilst markers of inflammation remained elevated, improvements in pre-Wallerian changes to corticospinal tracts were seen in 60% of the patients at 3 months follow-up by MRI. Dehghani et al. [16] also showed the safety of intraparenchymal injection of allogeneic placenta-derived MSC exosomes in five patients with a mean NIHSS of 17.6 and a follow-up of 3 months. This represents the current clinical trial data available as of January 10, 2023 (Table 1).

Table 1. Comparative analysis of key factors in the selected clinical studies

Delivery methods	Study	Study type	Study population	No. of cases	NIHSS score range	Source of stem cells	No. of injected cells	Time point of administration	Duration of follow-up	Improvement of observation indicators	Experimental constraints
Systemic delivery	Bang et al. [3]	Randomized, controlled, early phase II clinical trial	30–75 y.o.; MCA stroke	5	7–14	Autologous; BM	50 million; two times	At 32–41 days	52 weeks	Higher BI; lower mRS and NIHSS	Small sample size
	MASTERS-1 [4]	Randomized, double-blind, placebo-controlled, phase II clinical trial	18–83 y.o.; AIS	67	8–20	Allogeneic; BM	400/1,200 million	at 24–48 h	12 months	Higher BI; lower mRS and NIHSS	Small sample size; expansion of time window from 24–36 h to 24–48 h
	MASTERS-2 [4]	Randomized, phase III clinical trial	≥ 18 y.o.; AIS	Recruiting	Data not included	Allogeneic	1.2 billion	at 18–36 h	365 days	Ongoing	Data not included

Table 1. Comparative analysis of key factors in the selected clinical studies (*continued*)

Delivery methods	Study	Study type	Study population	No. of cases	NIHSS score range	Source of stem cells	No. of injected cells	Time point of administration	Duration of follow-up	Improvement of observation indicators	Experimental constraints
	J-REPAIR [6]	Randomized, double-blind, placebo-controlled, multicentre, early phase II clinical trial	≥ 20 y.o.; anterior circulation AIS	42	5–20	Allogeneic; dental pulp	100/300 million	within 48 h	366 days	Ongoing	Small sample size; proof-of-concept study-further studies required
	Law et al. [7]	Randomized, assessor-blinded, controlled, single-center, phase II clinical trial	30–75 y.o.; MCA stroke	9	10–35	Autologous; BM	2 million/kg body weight	within 2 months	12 months	No difference with control group	Small sample size
	STARTING-2 [8, 9, 12]	Randomized, prospective, open-label, controlled trial	30–75 y.o.; MCA stroke	39	6–21	Autologous; BM	1 million/kg body weight	within 90 days	3 months	No difference with control group	Small sample size; open-label design; short follow-up duration
	AMASCIS [10]	Randomized, double-blind, placebo-controlled, single-center, phase IIa pilot clinical trial	≥ 60 y.o.; AIS	4	8–20	Autologous; AD	1 million/kg body weight	within 2 weeks	24 months	Less AEs; lower NIHSS	Small sample size
Direct *in loco* injection	PISCES-2 [14]	Prospective, open-label, single-arm, multicentre study	> 40 y.o.; upper limb motor deficit after AIS	23	Arm score 2–4	Allogeneic; neural	20 million	at 2–13 months	12 months	Improved ARAT in 7 patients; BI in 8; mRS in 7	Small sample size; lack of control group; open-label design
	PASSIoN [15]	First-in-human, open-label, single-arm, single-center, early phase II, intervention study	Neonates (full-term) with MCA PAIS	10	Data not included	Allogeneic; BM	45/50 million	within 7 days	3 months	Improvements in pre-Wallerian changes to corticospinal tracts in 60% of patients	Small sample size; lack of control group; short follow-up duration
	Dehghani et al. [16]	Randomized, prospective, single-center, pilot clinical trial	Malignant MCA stroke; decompressive craniectomy candidates	5	11–25	Allogeneic; placenta-derived exosomes	2 mL (356 μg/mL)	within 48 h	3 months	Decreased mRS and NIHSS in 4 patients	Small sample size; short follow-up duration

y.o.: years old; BI: Barthel index; mRS: modified Rankin scale; AIS: acute ischemic stroke; AD: adipose tissue-derived

Conclusions and future perspectives

All of the above constitutes primary evidence of the relative safety of various modes of delivery of stem cells or their secreted exosomes to patients who have suffered from ischemic stroke. However, the heterogeneous nature of the individual studies together with the small numbers of individuals treated [with lack of Food and Drug Administration (FDA) or regulatory approval being a factor] does not support a strong hypothesis that such therapy has any significant efficacy. Considering the pathobiological complexity of the cellular and tissue associated events following stroke, it is likely that stratified, tailored, and targeted time sensitive approaches will need to be integrated into these therapies in order to provide meaningful improvements in outcome.

The concept of drug targeting and delivery to the brain is not new and Bruch et al. [17] summarized the use of liposomes for successful delivery of pH-sensitive drugs through the BBB, maintaining a longer potential half-life than directly delivered substances. Similarly, either MSCs themselves or their secreted vesicles could be magnetized, for example, by charging with iron oxide nanoparticles, and then targeted with magnets to the damaged part of the brain, although so far this has only been tested successfully in rodent models of MCAO [18]. However, to date, the evidence does not suggest significant penetration of MSCs through the BBB following systemic delivery, but paracrine effects from direct secretion, for example, of anti-inflammatory cytokines, in addition to polarized stimulation of the innate immune response, are likely to result in some beneficial modification to the stroke or penumbral micro-environment.

Regarding the heterogeneity of MSCs derived from different sources, there are small differences in general paracrine secretions, for example, comparing BM-derived, umbilical cord purified, and adipose tissue extracted cells. However, bigger differences are likely due to excessive passaging of cells to create the 'X' millions required in treatment protocols resulting in mutated aging cells. Further assessment of final therapeutics should be characterized for secretive patterns as MSCs are entirely capable of the pro-inflammatory phenotypical switch, for example, as seen in the adipose tissue of diabetic individuals [19].

In addition, MSCs are vehicles that can themselves be modified/primed with drug-loading to create an additional dimension for combinational type therapies, where release times have been shown to be up to 72 h. For example, Paudyal et al. [20] showed that MSCs loaded with a cyclin-dependent kinase 5 (CDK5) inhibitor known as CDK5 inhibitory peptide (p5; an anti-apoptotic 24-residue peptide that blocks p35-CDK5 aberrant phosphorylation), when delivered intracortical adjacent to temporarily induced MCAO, led to significant improvements in spatial learning, memory, and motor function over a period of 4 weeks (determined using the Morris water maze).

Specifically, whilst the mean infarct volume was not significantly reduced, the treatment also led to improvement in bilateral coordination and sensorimotor function (rotating pole), and asymmetry of forelimb usage (cylinder test). There was no effect on cutaneous sensitivity (adhesive tape removal test). Immunofluorescence staining with human cell-specific antibodies indicated a higher number of surviving transplanted cells in the peri-infarcted area of animals treated with human-adipose-derived MSCs (hADMSCs) + p5 compared with hADMSC only-treated or control animals, with a concomitant reduction in the number of phagocytic, annexin 3-positive cells.

Other options for longer-term release of pharmacologically active substances in larger amounts may even include micro-fragmented adipose tissue, where, for example, the release of paclitaxel was sufficient to perturb the growth of murine xenografted mesothelioma [21, 22].

When considering the maintenance/protection or recovery of peri-infarcted regions, neuronal plasticity and connectivity rely heavily on extracellular matrix (ECM) stability, and this is degraded and destabilized by proteinases and other molecules soon after stroke. A key molecule is hyaluronan, which plays the main structural role as a scaffold and is essential for remodeling and synaptic plasticity; hence, its breakdown into pro-inflammatory oligosaccharides by hyaluronidases after stroke must be addressed [23].

In addition, its potential as a drug delivery natural hydrogel amongst others, suggests it may have an important role in future acute stroke therapy [24, 25]. Jiang et al. [26] recently showed that hyaluronic acid-based hydrogels containing MSC-derived EVs, and implanted into the ischemic mouse brain, significantly

enhanced brain focal retention time (stabilizing the vesicles) and improved both angiogenesis and neurobehavioural recovery.

Thus, in conclusion, even though these possibilities hold promise, there is still a very long way to go before an effective therapy for individuals after stroke can be optimized, and a much more inclusive concerted approach is needed in order to facilitate appropriate larger scale clinical trials.

Abbreviations

BBB: blood-brain barrier

BM: bone marrow

CDK5: cyclin-dependent kinase 5

EV: extracellular vesicle

MCA: middle cerebral artery

MCAO: middle cerebral artery occlusion

MRI: magnetic resonance imaging

MSCs: mesenchymal stem cells

NIHSS: National Institutes of Health Stroke Scale

PAIS: perinatal arterial ischemic stroke

tPA: tissue plasminogen activator

Declarations

Author contributions

YP and MS equally contributed to: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical approval

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Consent to participate

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