

REVIEW ARTICLE

**Mesenchymal Stem Cell Therapy for Ischemic Stroke in Animal Model:
A Systematic Review**

Muhammad Agus Aulia^{1,2}, Keri Lestari^{1,3,*}, Ahmad Faried^{3,4}, Cynthia Retna Sartika⁵,
Andi Wijaya⁶, Achmad Adam⁴, Hanna Gunawan⁷, Rima Haifa⁵, Nabilla Farah Naura⁵,
Billy Yosua Costantin Pongajow⁵

¹Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM 21, Jatinangor 45363, Indonesia

²Department of Neurosurgery, Dr. Gatot Soebroto Central Army Hospital, Jl. Kwini No.1, Senen, Jakarta 10410, Indonesia

³Oncology and Stem Cell Working Group, Faculty of Medicine, Universitas Padjadjaran, Jl. Professor Eyckman No.38, Bandung 40161, Indonesia

⁴Department of Neurosurgery, Faculty of Medicine, Universitas Padjadjaran – Hasan Sadikin Hospital, Jl. Professor Eyckman No.38, Bandung 40161, Indonesia

⁵PT Prodia StemCell Indonesia, Jl. Kramat VII No.11, Jakarta, 10430, Indonesia

⁶Prodia Clinical Laboratory, Jl. Supratman No 43, Bandung 40114, Indonesia

⁷Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM 21, Jatinangor 45363, Indonesia

*Corresponding author. E-mail: lestarikd@unpad.ac.id

Received date: Jun 8, 2023; Revised date: Dec 5, 2023; Accepted date: Dec 8, 2023

Abstract

BACKGROUND: Stroke is one of many leading causes of death and disability worldwide. Despite decades of research, treatment for stroke recovery is still inadequate. Recently, mesenchymal stem cell (MSC) therapy is considered as one of the potential treatments for stroke due to their capabilities to repair or regenerate damaged tissues, as well as immunomodulators in inflammatory conditions. Many pre-clinical and clinical trials have shown MSC therapy feasibility, safety, and efficacy in treating stroke. However, evidence regarding the optimal treatment plan and factors that can improve stem cell functional outcomes in treating stroke needs to be further explored. Therefore, a systematic review was conducted.

METHODS: Recent literatures from 2015-2022 regarding stem cell therapies, specifically MSC on ischemic stroke, were collected from two reliable databases: PubMed and PubMed Central. Collected literatures

were properly applied to inclusion-exclusion criteria and appraised critically. Keyword strategies on databases were employed, including both medical subject headings (MeSH).

RESULTS: Five literatures from 726 were identified and used for systematic review. All animal model in the literatures were prepared to have middle cerebral artery occlusion. All studies indicated that MSC therapy is a safe and reliable procedure despite the variety of transplantation routes. No report of toxicity, rejection reaction, nor infection on MSC treated groups.

CONCLUSION: Stem cell sources, dosages, and delivery routes could be resourceful for future translational studies to ensure the safety and efficacy of MSC therapy for ischemic stroke.

KEYWORDS: ischemic stroke, mesenchymal stem cells, regenerative medicine, immunomodulator

Indones Biomed J. 2023; 15(6): 351-7

Introduction

Stroke is classified into two types: ischemic stroke and hemorrhagic stroke. Ischemic stroke is caused by the

obstruction of cerebral blood arteries, which is commonly caused by thrombosis or embolism.(1-4) Hemorrhagic stroke develops when cerebral blood vessels or aneurysms burst.(5-9) About 85% of strokes are ischemic, caused by a blockage in a brain-supplying artery, which results

in irreversible cell damage in the ischemic core, which is surrounded by a penumbra of surviving neurons. The rescue of the ischemic penumbra effects functional recovery and is the goal of current interventions.(7,10-15) Ischemic stroke complex cascade of events leads to the loss of neural tissue, neurons, and supporting structures, as well as initiating a local and systemic inflammatory response, causing damage, over a period of days.(8,16-18)

Additionally, although stroke is a disease of the vascular system, it causes a significant immune response. The immune response is involved in healing, but also includes a neuroinflammatory component that involves exacerbating the initial injury through destruction of neural tissue.(19-22) Stroke remains a common disease with limited treatment options. Available treatments provide little improvement in neurogenesis and recovery. Because of the limitations of currently available treatments, new treatments for stroke are needed.

Because of stem cells ability to self-replication and self-differentiation (23-27), stem cell therapy is recognized by its potential neurodegenerative capabilities in stroke patients such as mechanisms involving angiogenesis, neurogenesis, immune modulation, and even synaptic plasticity, which can result in both structural and functional improvement and regeneration of brain tissues. (6,8,28-30)

Currently, in clinical trials of patients with ischemic stroke, many types of stem cells are used and among them; bone marrow-derived mesenchymal stromal cells (BM-MSCs) is the most extensively researched due to its effects by immunomodulation and release of trophic factors that can promote angiogenesis, synaptogenesis, and neural precursors recruitment.(31-34) Other types of MSCs also include umbilical cord derived mesenchymal stem cell (UC-MSC) and adipose derived mesenchymal stem cell (AD-MSC), which shares their specific usefulness in ischemic stroke treatment; both in pre-clinical and clinical setting.(10,35-37) Thus, showing MSCs capabilities as an attractive novel approach to promote recovery in ischemic stroke treatment, further evidenced by encouraging results from many pre-clinical treatments.(35,38-41)

As the use of MSCs becomes favorable and is advancing to clinical stage, there is a need to better understand the different factors in effect during MSC treatment in ischemic stroke. Factors such as administration method and delivery route, dosage, cell type, as well as age and gender of stroke animals, needs to be addressed to see whether it has impacted the functional outcomes. Therefore, this systematic review was performed to evaluate the

efficacy of MSC therapy on ischemic stroke, based on the pre-clinical research.

Methods

Literature Screening

We conducted a systematic review of pre-clinical literature involving both graphical and narrative information. The protocol used to conduct this review was based on the “Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Research literatures reported in English between 2015-2022 were searched using PubMed and PubMed Central (PMC) with the keywords of “mesenchymal stem cell”, “MSC”, “ischemic stroke” and “therapy”. Then, the studies were further screened for animal models with clear functional outcomes and sample sizes. Meanwhile, genetically modified MSC and MSC with the combination of other treatment studies were excluded. The authors independently analyzed selected literature and tracked the data.

Literature Analysis, Data Extraction and Synthesis

We followed the recommendations of the PRISMA protocol above and extracted the data from the included study through standard data extraction form and included the following data: animal species, sample size, stroke animal model, source of MSCs, administration routes, dosage of transplanted cells or conditioned medium, gender of animal model, and functional outcome measures. Differences between authors were resolved through discussion.

Risk of Bias and Quality Assessment

The quality of the studies was assessed according to the STAIR guidelines, including: (1) manuscripts published in peer-reviewed journals; (2) random treatment assignments; (3) inclusion of sample size; (4) confirmed statement regarding animal welfare requirements compliance; (5) avoided neuroprotective anesthetics; (6) animals with relevant comorbidities are used; (7) statement declaring absence or presence of conflicts of interest; (8) statements describing control of temperature; (9) concealment of study allocations; and (10) blinded assessment outcomes. Criteria ranged from 1-10; with a point awarded for each reported criterion. The guideline was also used to determine the risk of bias in the included studies, using the same criteria and point awarded imposed on quality assessment. The PRISMA flow diagram can be seen in Figure 1.

Results

A total of 726 literatures were identified through database searching. Then 709 literatures were screened out, since the study in the literatures did not use animal model, did not have clear functional outcomes and did not have any information of sample size. After the screening, 17 literatures were left and put into the selection process (Figure 1). Finally, 5 literatures were included after the exclusion of 12 literatures. The 12 literatures were excluded since the studies reported were applying genetically modified MSC or MSC with the combination of other treatments.

From 5 included literatures (Table 1), 4 literatures reported studies using allogenic MSC while 1 literature using conditioned media (CM) of MSC. The allogenic MSC sources were various, included bone marrows (n=2), umbilical cord (n=1), and olfactory mucosa (n=1). While CM-MSC was derived from human embryonic MSC.

For the animal model, Sprague-Dawley and Wistar rats with middle cerebral artery occlusion (MCAO) were used. MCAO procedure was performed differently, one study using Laser Doppler Flowmetry (LDF) (42), and the rest of the study used intraluminal insertion of 90 minutes-monofilament nylon suture from the external carotid artery (ECA) to the internal carotid artery (ICA) (43-46).

Number of administered stem cells were varied, 1×10^5 , 1×10^6 , and 5×10^6 , while volume of administered CM was 5 mL. The routes of stem cell administration were intraarterial (IA) and intravenous (IV), and intracerebral (IC). For the CM-MSC, the route of administration was intracerebroventricular (ICV).

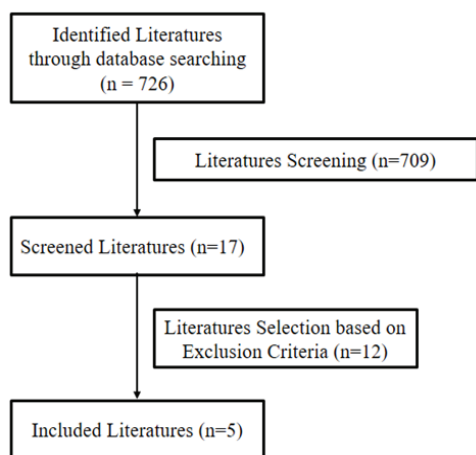


Figure 1. PRISMA flow diagram of literature identification, screening and selection. Screening and selection processes were performed according to the criteria listed in the Methods section.

Risk of Bias Assessment

The risk bias across the 5 studies were moderate at best. The animal characteristics, interventions, and functional outcomes were clearly described in the study. All the actual values and side effects were reported. However, 4 out of 5 studies did not clearly declare the study design used in the experiment. Overall, the main outcome measures used were valid and reliable.

Study Outcomes

All the 5 studies included in this review were able to be included in the quantitative analysis. The post-therapy neurological outcomes were measured through behavior, motor, and biochemical expression assessments. The comparison was undertaken between rats with and without stem cell intervention at the time when the baseline neurological functions were measured.

Regarding the behavior assessment, the 5 studies reported that most of the rats displayed the most robust significant improvements in Modified Neurological Severity Scores (mNSS) at 3 and 7 days after stem cells transplantation compared to stroke rats which did not receive interventions. Other behavior assessments were also conducted, for instance somatosensory test, which showed a significant recovery after MCAO. Fewer apoptotic cells were observed and more immunoreactive cells were found in the ischemic boundary area.

Regarding the motor assessment, these 5 studies reported that there were loss of infarct volumes and glial scar formations. An increase of subventricular counts of proliferating neuroblasts, and cerebrovascular growth in ischemic penumbra regions were observed. The results also indicated that stem cells administration could significantly reduce weight loss, mortality rate, and cerebral edema. In addition, injection of stem cells could also restore synaptic markers in MCAO rats up to their normal levels.

Meanwhile, regarding the biochemical expression assessment, all studies reported that stem cell transplantation could reduce the expression of Calcineurin (CaN) after ischemic stroke and improves the functional outcome and normalizes oxidative parameters. In addition, bFGF and SDF-1 α levels of the infarct cortex were highly elevated compared to the rats that did not receive any intervention.

Discussion

This systematic review regarding pre-clinical research shows the potential effects of MSC in improving clinical

Table 1. Summary of 5 literatures reviewed in this study.

Species	Sample Size	Stroke Model	MSC source	Route of Administration	Dosage	Gender	Functional Outcome Measures	References
Sprague-Drawley Rat	40 rats	MCAO	Allogenic OM-MSCs	Intravenous (IV)	5x10 ⁶	Male	<ul style="list-style-type: none"> - MSC represses elevation in GOLPH3 caused by cerebral IRI. - MSC PEDF upregulated PEDF, and in turn, upregulated phosphorylation of Akt in OGD/R-injured N2a cells through PEDF-R integration. - MSCs mitigated GA stress response, suppressed autophagy, and partially inhibited apoptosis. 	43
South Sprague-Drawley Rat	13 rats	MCAO	Allogenic hUC-MSCs	Intravenous (IV)	1x10 ⁶	Male	<ul style="list-style-type: none"> - MSCs IV transplantation fell short in most aspect in ischemic stroke MSC therapy. - MSCs IV is reported to be safe but lacking significant functional recovery. - MSCs IV injection can get the cell stuck in lungs, liver, or spleen; limiting effects of engraftment and migration. 	44
Wistar Rat	82 rats	MCAO	Allogenic BM-MSCs	Intracerebral (IC)	1x10 ⁶	Male	<ul style="list-style-type: none"> - MSCs IC transplantation of MSC significantly improved functional - MSC effects remain viable; presumably through paracrine reverberations and cell-to-cell exchanges with host brain in terms of brain repair. - MSCs IC is more effective; requiring lower doses and preventing embolism at subacute phases of stroke; while providing significant - MSC reduce pro-inflammatory response, glial scarring, spur SVZ neurogenesis and peri-infarct angiogenesis. 	45
Wistar Rat	114 rats	MCAO Occlusion	CM hESC-MSC (RH6-MSCs)	Intraarterial (IA) ventricular (ICV)	5 mL	Male	<ul style="list-style-type: none"> - Reduced infarct volumes and high levels of MSCs migration towards ischemic cortex. - Elevated levels of bFGF and SDF-1a neurotrophic factors; during MSCs migration. - Higher functional recovery in rats behaviour. 	46
Sprague-Drawley Rat	45 rats	MCAO	Allogenic BM-MSCs	Intraarterial (IA)	1x10 ⁵	Female	<ul style="list-style-type: none"> - CM administration significantly reduce cerebral edema and infarct volume in MCAO rats; improved neurological deficit following MCAO as well. - Other MSCs such as AD-MSC administered ICV reduced infarct volume and ameliorate rats neurologic deterioration post. - CM is shown to significantly increase mean body weight of MCAO mice from day 3 postinjury. BM-MSC shows similar trait as well. - CM treatment greatly decreased mortality rate in mice. - CM administration elevated GAP-43 and SYP in rat stroke model; contributing to functional improvement during recovery period. - CM administration elevated CREB phosphorylation in MCAO; a mechanism involved in neuroprotection and improved neural function. 	42

MCAO: Middle cerebral artery occlusion; BM-MSC: Bone marrow derived-mesenchymal stem cell; OM-MSC: Olfactory mucosa derived-mesenchymal stem cell; hUC-MSC: Human umbilical cord derived-mesenchymal stem cell; CM: Conditioned medium; hESC-MSC: Human embryonic stem cell derived-mesenchymal stem cell; PEDF: pigment epithelium-derived factor; OGD/R: oxygen-glucose deprivation/reoxygenation; GOLPH3: Golgi phosphoprotein 3; SVZ: subventricular zones; bFGF: basic fibroblast growth factor; GAP-43: growth-associated protein-43; SYP: synaptophysin; CREB: cAMP response element-binding protein; CaN: calcineurin

outcomes after experiencing stroke and the understanding of MSC mechanism in ischemic brain. The main finding is that improvements in motor outcome were shown after MSC administration, despite the variety of secreted tropic factor compositions, anti-inflammatory cytokines, and another paracrine factors by each source of MSC. MSC treatment also showed infarct volume reduction and improvements of neurological deficit, sensorimotor and cognitive performance in arterial ischemic stroke neonatal animals. These results reinforce the neuroprotective role of MSC therapy in treating ischemic stroke.(47,48)

MSC could act as neuroprotectors by reducing apoptosis in the early phase of the ischemic brain. The transplantation of olfactory mucosa-derived MSC has been shown to suppress autophagy and reduce apoptosis by modulating golgi apparatus stress response through the PEDF-PI3K/Akt/mTOR Pathway.(43) Meanwhile the role of MSC in promoting endogenous neuro-angiogenesis was also highlighted. Significant increase of neural progenitor cells coupled with the elevation of angiogenesis in ischemic penumbra was reported.(44) Secreted neurotrophic factor by MSC is considered as a part of the underlying mechanism. There was an increase of new neural progenitor cell in subventricular zone and loss of apoptosis by upregulating FGF and VEGF expressions through trophic factor secretion.(49) And the elevation of neurotrophic factors namely hFGF and SDF-1alpha were correlated with reduced infarct volume in MSC transplanted rats.(45)

The dosages and routes of MSC administration used in this research were varied. The IV route was preferable due to its simple and less complicated procedure. However, MSC administration via IV shows a significant number of MSC were trapped in the lung, liver, and spleen. Therefore, it limited the MSC migration to the ischemic brain and required a higher MSC dose compared to other routes. The IV MSC-transplanted group had lower clinical improvement compared to IC MSC-transplanted group. IA was another option of administration which offered MSC migration through a disrupted blood brain barrier directed to ischemic brain.(44) Good motor outcomes was achieved after the intraarterial injection of 1×10^5 - 10^6 MSC.

MSC thrombo-emboli risks should be aware, since it could make a more devastating condition.(42,45) IC-transplanted MSC triggered significant motor improvement, inflammation reduction, and neuro-angiogenesis increase compared to IV-transplanted MSC groups.(44) In addition, a lower MSC dose was also utilized in this procedure, since the MSC was delivered directly inside the brain tissue penumbra.(44)

All studies indicated that MSC therapy is a safe and reliable procedure despite the variety of transplantation routes. No report of toxicity, rejection reaction, nor infection on MSC treated groups.(42-46) There was also no tumor formation in 1-year follow up after MSC transplantation. However, stem cell transplantation cannot certainly re-integrate the neural circuits for functional neurorestoration. Therefore, further research to investigate the mechanism of stem cell transplantation in this issue is highly necessary.(50)

Conclusion

MSC therapy could ameliorate ischemic brain condition of stroke animal model by improving the behavior and motor function. It is strengthened by the evidence of the increase of neuro-angiogenesis in the ischemic brain penumbra. This systematic review has highlighted stem cell sources, dosages, and delivery routes, which could resourceful for future translational studies from bench to bedside to ensure the safety and efficacy of MSC therapy for ischemic stroke.

Authors Contribution

MAA, KL, AF and, CRS were involved in the conceptualization of the study. MAA, KL, AF, CRS, AW, AA, and HG took parts in the preparation of the methodology. MAA, KL, AF, CRS, AW, AA, and HG conducted the data curation. RH, NFN, and BYCP analyzed the data with software; while MAA, KLD, AF, and CRS did the formal analysis. MAA, RH, NFN, and BYCP prepared the original draft of the manuscript, as well as did the review and editing of the manuscript. KL, CRS, AW, AA, and HG supervised the whole study. All authors have read and agreed to the published version of the manuscript.

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