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Effects of stem cell therapy on preclinical stroke

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ABSTRACT
Stroke, particularly ischemic stroke, is a leading cause of global mortality and disability. It is caused by blood flow obstruction and reduced oxygen delivery to brain tissue. Conventional treatments, such as tissue plasminogen activator (tPA) and mechanical thrombectomy (MT), have limited efficacy in repairing neural damage and carry risks of adverse effects. As a result, stem cell therapies, including mesenchymal stem cells (MSCs), have emerged as promising approaches for enhancing neural recovery and offering neuroprotection in ischemic stroke management. MSCs offer multifaceted benefits, such as reducing inflammation, protecting neurons, and promoting angiogenesis and neurogenesis. Recent evidence highlights the importance of MSC secretomes–extracellular vesicles (EVs) and exosomes rich in neuroprotective factors, such as microRNAs, proteins, and cytokines. These bioactive molecules demonstrated considerable efficacy in preclinical models by reducing neuroinflammation, preserving neurovascular integrity, and promoting cellular repair in ischemic environments. Preclinical *in vitro* and *in vivo* studies demonstrate the potential of the MSC secretomes to restore brain function after ischemic stroke. This is achieved by enhancing neuronal survival through mechanisms such as angiogenesis or vascular recovery, neuroprotection including modulation of immune or inflammatory responses, apoptosis, and autophagy, and promoting post-stroke neurogenesis. This review explores the translational challenges and future potential of integrating conventional ischemic stroke therapies with stem cell-based or cell-free approaches. The present study synthesizes current insights into the role of MSC-derived secretomes from both *in vitro* and *in vivo* studies.
Keywords: Ischemic Stroke, Stem Cells, Preclinical, *In Vitro*, *In Vivo*.

Introduction

Stroke remains a leading cause of death and chronic disability worldwide, with a particularly pronounced burden in developed countries. It is characterized by abrupt cessation of cerebral blood flow caused by ischemic events (blood clots) or hemorrhagic events (ruptured blood vessels). These conditions often result in permanent brain injury and decreased motor and cognitive function (Katan and Luft, 2018). Stroke can be broadly classified into two main types: ischemic and hemorrhagic. Ischemic strokes account for 80%–87% of cases and occur due to reduced blood flow and oxygen delivery, typically due to arterial blockage. Neuronal damage in the brain caused by ischemic injury complicates treatment for patients with this condition (Chang, 2020). Hemorrhagic stroke, occurring in 10%–15% of cases, involves blood vessel rupture, causing tissue damage and cell death. The clinical manifestations of stroke vary according to location, type, and severity (An *et al.*, 2017).

The clinical manifestations of stroke depend on the brain region, in which blood circulation is disrupted. Common symptoms include body weakness, speech difficulties, vision loss, dizziness, and episodes of falling. Hemorrhagic stroke often causes more pronounced dizziness than ischemic stroke (Chugh, 2019). Diagnostic tools, such as the 6S test, sudden onset, slurred speech, side weakness, spinning (vertigo), severe headache, and second (time of onset), are used to identify strokes. Additionally, the BEFAST approach emphasizes symptoms, such as balance issues, eye (vision) problems, facial drooping, arm weakness, and speech difficulties (Chen *et al.*, 2021).

The primary objective of treatment for ischemic stroke is to rapidly reestablish cerebral blood circulation, with reperfusion being the cornerstone of management. Current interventions include intravenous tissue

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plasminogen activator (tPA), mechanical thrombectomy (MT), and pharmacological agents, such as aspirin, clopidogrel, and statins (Yaqubi and Karimian, 2024). Intravenous tPA is the gold standard for ischemic stroke therapy, and it is most effective when administered within the first 4.5 hours following symptom onset because its efficacy significantly declines beyond this window (Singh *et al.*, 2020). However, tPA is associated with the risk of intracerebral hemorrhage, which may increase mortality. For cases involving large artery occlusion with substantial clot burden or when tPA is administered outside the optimal timeframe, MT is a viable alternative (Dong *et al.*, 2020).

Given the limitations of tPA and MT in promoting neural regeneration, therapies with neuroprotective neurogenic potential are needed. Cellular therapies have emerged as promising options for managing ischemic stroke, offering the potential to enhance patient recovery outcomes (Katan and Luft, 2018; Borlongan, 2019). Emerging evidence suggests that stem cell therapy can effectively improve neurological function in patients with ischemic stroke. Its potential lies in enhancing the brain's neuroprotective mechanisms and aiding repair processes through immunomodulation and modulation of neuronal, vascular, and glial cell functions (Ejma *et al.*, 2022). Stem cells have the potential to restore damaged brain tissue and mitigate neuroinflammation. They are distinguished by their ability for self-renewal and multipotency, which allows them to differentiate into multiple cell types (Cha *et al.*, 2024). Several stem cell varieties—mesenchymal stem cells (MSCs), bone marrow mononuclear cells (BMMCs), neural stem cells (NSCs), and induced pluripotent stem cells (iPSCs)—have been evaluated using diverse delivery routes, such as intravenous, intra-arterial, and intracerebral methods, over durations ranging from a few hours to several months (Houkin *et al.*, 2024).

The objective of this review article was to provide a comprehensive understanding of the potential role of stem cell therapy, specifically MSCs and their derived secretomes, in enhancing post-stroke recovery, particularly in ischemic stroke. This study aimed to elucidate the underlying biological mechanisms through which MSCs exert neuroprotective effects and promote brain function recovery, focusing on the secretion of bioactive molecules, such as exosomes and extracellular vesicles (EVs) that modulate inflammation, apoptosis, and neurogenesis. By synthesizing findings from preclinical *in vitro* and *in vivo* studies, this review aims to offer insights into the translational challenges associated with integrating conventional stroke therapies with stem cell-based or cell-free approaches while also highlighting the preclinical potential of these therapeutic strategies for improving stroke recovery. The primary expectation is to provide a clear guide on the efficacy of MSC-derived secretomes in accelerating post-stroke repair, thereby paving the way for the development of more effective and safer therapeutic options in stroke management, with a focus on the biological aspects and translational hurdles in research and application.

Pathophysiology of stroke

Ischemic stroke results from cerebral artery obstruction by a mobile embolus, which may be cardiogenic, artery-to-artery embolism, or vascular stenosis. This obstruction disrupts blood flow to the brain, leading to significant functional and neurological deficits (Shehjar *et al.*, 2023). The resulting vascular occlusion leads to hypoxia, depriving downstream tissues of oxygen and glucose. Two distinct regions are affected: the ischemic core, where blood supply is critically insufficient for cell survival, and the ischemic penumbra, where cells remain viable for a limited time and recover if blood flow is promptly restored (Sun *et al.*, 2024). Prolonged hypoxia and glucose deprivation deplete ATP levels, triggering membrane depolarization and excitotoxicity driven by excessive glutamate release. Overactivation of glutamate receptors causes an influx of calcium ions (Ca2+), which enhances the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These molecules damage lipids and DNA while releasing inflammatory mediators. Mitochondrial impairment is frequently involved in this process, leading to cytochrome C release and programmed cell death (apoptosis) (Salaudeen *et al.*, 2024).

Several pathological events can exacerbate ischemic stroke, including inflammation, energy failure, disrupted homeostasis, acidosis, increased intracellular calcium levels, excitotoxicity, and free radical toxicity. Cytokines released during the immune response contribute to cytotoxicity by activating the complement system and compromising the blood–brain barrier's integrity. Additionally, glial cell activation, oxidative stress, and leukocyte infiltration further intensify stroke-related damage (Kuriakose and Xiao, 2020). Stroke is linked to various risk factors that can be classified as modifiable (e.g., dietary habits, preexisting comorbidities) or nonmodifiable (e.g., age, ethnicity). These factors also vary by time frame: short-term triggers (such as infections, sepsis, and psychological stress), medium-term contributors (including hypertension and hyperlipidemia), and long-term determinants (such as gender and ethnicity). It should be noted that these risk factors may differ significantly between younger and older populations (Boehme *et al.*, 2017; George, 2020; Yoon and Bushnell, 2023).

Mesenchymal stem cells (MSCs)

Cell-based therapies, particularly those using stem cells, offer prospective treatments for ischemic stroke. Stem cells can regenerate damaged neurons and exert neuroprotective effects on surviving neuronal cells, thereby accelerating neurological recovery (Rahimi Darehbagh *et al.*, 2024). These neuroprotective mechanisms include the inhibition of stroke-related pathways, such as neuroinflammation, blood–brain barrier (BBB) disruption, and apoptotic cell death,

while simultaneously promoting angiogenesis and neurogenesis (Xu *et al.*, 2017b).

Several types of stem cells have been explored for stroke therapy, including MSCs, bone marrow stem cells (BMSCs), multilineage-differentiating stress-enduring (Muse) cells, and neural stem or progenitor cells. These cell types have exhibited their ability to enhance neurogenesis, promote angiogenesis, facilitate cell migration, and reduce neuroinflammation (Chrostek *et al.*, 2019; Markowska *et al.*, 2023). Stem cells can be sourced from four main sources: (1) embryonic tissues (Weissman, 2015); (2) fetal tissues, including the (Rosner *et al.*, 2023) placenta (amnion and chorion), amniotic fluid, and umbilical cord (Wharton's jelly, cord blood) (Kargozar *et al.*, 2018; Pulido-Escribano *et al.*, 2022; Maraldi and Russo, 2022); (3) adult tissue niches, such as adipose tissue, bone marrow, skeletal muscle, skin, and blood (Visvader and Clevers, 2016; Zou *et al.*, 2016); and (4) iPSCs, derived from somatic cells that are genetically reprogrammed (Rony *et al.*, 2015; Cerneckis *et al.*, 2024). The International Society for Cellular Therapy defines MSCs based on three essential criteria: they must adhere to plastic substrates in standard culture conditions; express certain markers such as CD73, CD90, and CD105 while lacking expression of markers such as CD14, CD34, CD45, CD11b, CD79α, CD19, or HLA Class II; and demonstrate the ability to differentiate into osteoblasts, chondroblasts, and adipocytes under appropriate conditions (Ullah *et al.*, 2015; Utama *et al*., 2024).

Stem cells are categorized into three types: embryonic stem cells (ESCs), adult stem cells, and iPSCs (Ullah *et al.*, 2015). While adult stem cell therapies for ischemic stroke are promising, their precise therapeutic mechanisms remain poorly understood. ESCs and iPSCs can regenerate neurons in infarcted regions, but achieving effective neuronal replacement to restore functionality remains challenging (Bang *et al.*, 2016). Additionally, ESC and iPSC therapies carry a higher risk of tumorigenesis. Preclinical studies have shown that many transplanted cells do not survive beyond a few weeks after transplantation (Duan *et al.*, 2021). Notably, the limited survival of transplanted cells may be an advantage of stem cell therapy because it reduces the likelihood of unregulated stem cell proliferation (Fernández-Susavila *et al.*, 2019).

Adult stem cells, particularly MSCs, are considered a more promising alternative for stroke treatment because of their ability to release bioactive compounds such as trophic factors and extracellular vesicles (EVs). EVs are tiny membrane-enclosed particles typically sized between 0.1 and 1 μm that are discharged from the cell surface to target injured brain regions. These substances can promote neurogenesis, angiogenesis, and synaptogenesis (Lai *et al.*, 2011; Song *et al.*, 2013). Furthermore, MSCs are believed to play several critical roles, such as reducing inflammation and mitigating scar formation, enhancing autophagy, normalizing the microenvironment and metabolic profile, and potentially restoring damaged cells across various neurological conditions (Shin *et al.*, 2014; Yamauchi *et al.*, 2015).

Secretome mesenchymal stem cells (MSCs)

MSCs secrete various substances into their culture medium, collectively known as the secretome. The secretome comprises soluble factors such as growth factors, cytokines, chemokines, and extracellular vehicles (EVs) (Ferreira *et al.*, 2018; Foo *et al.*, 2021). Key growth factors commonly found in stem cell secretomes include vascular endothelial growth factor (VEGF), transforming growth factor beta-1 (TGF-β1), nerve growth factor (NGF), insulin-like growth factor 1 (IGF-1), and brain-derived neurotrophic factor (BDNF) (Cunningham *et al.*, 2018). Chemokines typically present in MSC secretomes include stromal cell-derived factor-1 (SDF-1 or C-X-C motif chemokine ligand (CXCL)12) and C-C motif chemokine ligand (CCL)2, along with cytokines such as interleukin (IL)-6, CD4+ memory T cells, and Th2 cytokines such as IL-4, IL-5, IL-6, IL-13, IL-8, and tumor necrosis factor-alpha (TNF-α)) (Han *et al.*, 2022; Dumingan *et al*., 2024).

Stem cell-based treatments, particularly with MSCs, have demonstrated promising results in preclinical ischemic stroke research. These treatments include improving neurological deficits, reducing infarct volume, and providing neuroprotective benefits (Zhang *et al.*, 2021a). Numerous studies have also highlighted the therapeutic potential of MSC secretomes, focusing on their roles in immunomodulation, inflammation reduction, neuroprotection, neurotrophy, apoptosis prevention, angiogenesis regulation, and overall tissue regeneration (da Silva *et al.*, 2023). Initially, MSCs were thought to repair damaged tissues through direct cell replacement. However, it is now widely recognized that their regenerative effects are primarily mediated by paracrine mechanisms (Cunningham *et al.*, 2018). The substances secreted by MSCs play a vital role in the recovery of injured tissues (Asgari Taei *et al.*, 2022).

Recent studies have indicated that preconditioning MSCs can alter their secretory profile, thereby enhancing the therapeutic potential of their secretome. Common *in vitro* preconditioning techniques include the exposure of MSCs to hypoxic environments, proinflammatory signals, and three-dimensional culture systems (Kahrizi *et al.*, 2023). These methods modulate MSC secretions by stimulating or inhibiting the release of factors with anti-inflammatory, immunomodulatory, antitumor, and regenerative properties, depending on the stimuli. For instance: a) hypoxic conditions enhance the synthesis of growth factors and anti-inflammatory compounds; b) proinflammatory stimuli stimulate the release of immunomodulatory and anti-inflammatory molecules; and c) three-dimensional culture systems increase the yield of anti-cancer and anti-inflammatory

factors compared with traditional monolayer cultures (Madrigal *et al.*, 2014).

The involvement of the MSC secretome in enhancing recovery in preclinical models of stroke

In recent decades, numerous models have been developed to address the diverse challenges of ischemic stroke, accounting for its varied etiologies and clinical presentations. These models aim to elucidate the mechanisms behind underlying ischemia and facilitate the discovery of novel therapeutic agents for ischemic stroke treatment (Fluri *et al.*, 2015). However, despite identifying key pathophysiological processes, only a small number of experimental protective agents have been successfully applied in clinical settings (Amado *et al.*, 2022). This gap underscores the discrepancies between the actual conditions of patients with stroke and preclinical ischemic stroke models. Therefore, it is essential to recognize the advantages and disadvantages of each stroke model to select an experimental system that aligns with specific research objectives and elements of interest (Jickling and Sharp, 2015).

Preclinical in vitro test results for stroke following MSC therapy

In vitro models are commonly used in ischemic stroke research, often involving cocultures of astrocytes and endothelial cells (Wevers *et al.*, 2021). These systems replicate key aspects of the blood–brain barrier (BBB) and provide insights into cellular interactions under ischemic conditions (Van Breedam and Ponsaerts, 2022). Some *in vitro* models use primary cultures obtained from animal brain tissue to study the effects of treatments on specific brain regions, such as cortical neurons. These models also facilitate investigations of cellular responses to ischemic-like conditions, including hypoxia and glucose deprivation. Furthermore, they allow researchers to explore the molecular pathways associated with cell death, such as necrosis, apoptosis, and autophagy. *In vitro* methods are often used alongside *in vivo* studies to provide deeper insights into human ischemia (Sommer, 2017; Barthels and Das, 2020). A common *in vitro* approach involves brain tissue slices, which are thin sections (approximately 400 micrometer) of brain tissue that facilitate detailed examination of neuronal circuits. Furthermore, organotypic culture methods that integrate brain slice cultures and primary cell cultures are used to study *ex vivo* brain tissue from young animals. Primary cell cultures, including brain endothelial cells (BEC) and glial cells, are also widely used in these *in vitro* models to investigate ischemic mechanisms (Holloway and Gavins, 2016; Singh *et al.*, 2022).

The most common *in vitro* method for simulating ischemic conditions, such as stroke, is oxygen–glucose deprivation (OGD). This method involves the complete removal of oxygen and glucose from the cellular environment (Trotman-Lucas and Gibson, 2021; Amado *et al.*, 2022). Ischemic conditions can also be replicated using chemical or enzymatic agents that inhibit metabolic activity. In the OGD model, a balanced O_2/CO_2 medium is replaced with an N_2/CO_2 medium to induce hypoxia. During this process, oxygen is substituted with nitrogen, and glucose is eliminated from the medium (Babu *et al.*, 2022). Cell cultures are used in this model to study cellular responses under ischemic conditions. Hypoxia during OGD can be induced using hypoxia chambers, anaerobic containers, incubators with hypoxic gas supplies, or anaerobic chambers equipped with palladium catalysts to remove oxygen via water formation. Among these, hypoxia chambers with gas supply are the most widely used approach for creating hypoxic environments (Sommer, 2017). The research findings related to the use of MSC therapy in in vitro models are presented in Table 1.

Table 1. Overview of research examining the effectiveness of MSC therapies in preclinical *in vitro* models of cerebral ischemia and the roles of secretome components.

Cell used	MSC therapy	Stroke model	Dose	Timing post-stroke	Marker analysis	Potential role of the secretome	References
Primary cortical astrocytes (Wistar rat brains)	Rat MSC-Exos	OGD/R 6 h/24h	(−)	48 hour	GLT-1, miR-124, and mTOR	Neuroprotection (autophagy)	(Huang *et al.*, 2023)
HBMECs	BMSCs	OGD/R 4h/24 h	(−)	48 hour	uPA/uPAR SDF-1α/CXCR4	Angiogenesis (migration)	(Fang *et al.*, 2022)
Mouse BV-2 microglial cells	Mice NSC-Exos	OGD/R 4h/12h	(−)	24 hour	ZEB1, GPR30, TLR4, NF-κB	Neuroprotection (inflammation)	(Peng *et al.*, 2023)

(Continued)

Cell used	MSC therapy	Stroke model	Dose	Timing post-stroke	Marker analysis	Potential role of the secretome	References
HUVECs, PC12 cells, and rBMDM	Human BMCS with IONP	Hypoxia (1% O_2) 24 h	40 µg/ml	48 hour	Annexin V-FITC, Bcl-2, and Bax Ang-1, FGF2, HGF, and VEGF levels	Neuroprotection (apoptosis) Angiogenesis	(Kim *et al*., 2020)
bEnd.3	BMSC-EVs	4 or 6 h OGD	~2× 10^10 cells	4 or 6 hours	Caveolin-1, Claudin-5	Angiogenesis	(Li, Liu, *et al.*, 2023)
Mouse cortical cells	hUC-MSC	6h OGD	(-)	24 hour	AKTNrf2 IL-4, IL-10, TGF-β1, GSK-3β	Neuroprotection Neuroprotection (antioxidant) Neuroprotection (inflammation)	(Li *et al.*, 2023a)
Rat PC12 and HEK 293T cells	Rat BMSC-Exos	OGD/R 2 h/24 h	20 mg/ml	24 hour	miRNA-193b-5p, AIM2, GSDMD-N, Cleaved Caspase-1, IL-1b/IL-18	Neuroprotection (inflammation)	(Y. Wang *et al.*, 2023)
N2a cells and primary cultured neurons	Mice BMSC-Exos	OGD/R 2 h	10-80 µg/ml	48 hour	KLF4	Neuroprotection (inflammation)	(Q. SouthS. Wang *et al.*, 2023)
bEnd.3 cells, Mouse primary astrocytes	Human UCMSCs	OGD/R 2h/45 min	200 µg	3 hour	TLR4/NF-κB and miRNA-125b-5p	Neuroprotection (inflammation)	(Qiu *et al.*, 2022)
BV2 cells	hPMSCs	In Vitro after 4 h of treatment.	100 µl	2 days	CXCL12/CXCR4	Neurogenesis (migration)	(Li *et al.*, 2021)
HUVECs and SH-SY5Y cells	3D spheroids of UC-MSCs	oxygen-glucose deprivation (OGD) model	(−)	14 days	BDNF, IGF-1 VEGF	Angiogenesis	(Hsu *et al.*, 2021)
HTT2 cell line	hADSCs	OGD/R 10h/24 h	1×10^{10} particles/ml	24 hour	Bax/Bcl-2 miR-146a/miR21	Neuroprotection (apoptosis)	(Jang *et al.*, 2024)
BV2 cells	hUMSC-Exos	OGD/R 6 h/24h	n/a	24 hour	iR-146a/miR21, IRAK1/TRAF6, IL-6, TNF-α, IL-1β, NFκB, Toll-like receptor (TLR)	Neuroprotection (inflammation)	(Z. Zhang *et al.*, 2021)
HUVECs	iMSC-sEV	OGD 8h	1×10^{9} particles/ml	24 hour	STAT3 LC3-II/LC3-I, Beclin-1, P62	Angiogenesis Neuroprotection (autophagy)	(Xia *et al*., 2020)
PC12 cells	hNSCs	OGD 6h	(−)	24 hour	PRDX6	Neuroprotection (antioxidant)	(Tang *et al.*, 2021)

MSC therapies are advancing ischemic stroke treatment by delivering exosomes (Exos) and extracellular vesicles (EVs) that engage specific cellular pathways to confer neuroprotection, support angiogenesis, and mitigate inflammation. In astrocytes derived from Wistar rat brains, MSC-derived exosomes exert neuroprotective effects under ischemic conditions by upregulating GLT-1 via the miR-124/mTOR pathway, promoting autophagy, and reducing neuronal stress during OGD/R injury (Huang *et al.*, 2023). Similarly, bone marrow-derived MSCs (BMSCs) modulate key pathways in human brain microvascular endothelial cells (HBMECs), specifically uPA/uPAR and SDF-1α/CXCR4, to promote angiogenesis and neurogenesis—essential for vascular repair post-stroke (Fang *et al.*, 2022). Microglial BV-2 cells benefited from a NSC-derived exosomes, which contained ZEB1, to upregulate GPR30, suppress TLR4/NF-κB signaling, and consequently reduce inflammation (Peng *et al.*, 2023). Further, MSC therapies enhance blood–brain barrier (BBB) integrity, as shown in bend. Three endothelial cells and primary astrocytes were treated with UCMSC-derived exosomes rich in miR-125b-5p, which blocked the TLR4/NF-κB pathway and safeguarded BBB structure and function (Qiu *et al.*, 2022).

MSC therapies also play a crucial role in apoptosis regulation and antioxidant defense. For example, under hypoxic conditions, BMSC therapies combined with iron oxide nanoparticles (IONP) in HUVECs and PC12 cells activated VEGF, Bcl-2, and TGF-β, which collectively inhibited apoptosis and reduced cellular injury (Kim *et al.*, 2020). Moreover, adipose-derived MSCs (hADSCs) promoted HTT2 cell survival post-OGD/R by adjusting factors that promote or inhibit apoptosis Bax/Bcl-2 and miR-146a/miR-21, thus supporting neuroprotection through apoptosis inhibition (Jang *et al.*, 2024). Notably, human NSC-derived exosomes in PC12 cells activated the antioxidant enzyme PRDX6, thereby combating oxidative stress and enhancing cell survival under ischemic conditions (Tang *et al.*, 2021).

Other models, such as PC12 and HEK293T cells, respond to rat BMSC-exosomes containing miRNA-193b-5p with reduced inflammatory responses (Wang *et al.*, 2023b). Importantly, UC-MSC-derived three-dimensional spheroids produce paracrine factors such as BDNF, VEGF, and IGF-1 in HUVECs and SH-SY5Y cells—providing neuroprotection and support angiogenesis and anti-apoptotic mechanisms (Hsu *et al.*, 2021). These studies, alongside others focusing on pathways such as STAT3 and miR-125b-5p, underscore the diverse molecular pathways that MSC therapy can target to enhance post-stroke recovery and therapeutic efficacy across models (Xia *et al.*, 2020; Qiu *et al.*, 2022).

The therapeutic potential of MSC-derived secretomes—including microRNAs and cytokines—highlights their capability to address multifaceted aspects of stroke pathology. By targeting inflammation, apoptosis, oxidative stress, and vascular repair, MSC-based therapies provide a promising platform for developing advanced ischemic stroke interventions and supporting neuronal recovery. This growing body of evidence underscores MSC therapies as a powerful approach within the field of tissue regeneration, paving the way for clinical applications in ischemic stroke recovery and beyond.

Preclinical in vivo testing in stroke with MSC therapy

While numerous animal species have been used in preclinical ischemic stroke studies, rodents are primarily favored for ischemic stroke research. This preference is due to their lower costs and the similarity of their cranial blood circulation to that of human neurovascular structures (Narayan *et al.*, 2021). Additionally, rodents offer practical advantages in controlling ischemic models, including precise manipulation of occlusion severity, duration, and location. Other species, including rabbits, pigs, nonhuman primates, and nonprimate mammals, are also used in ischemic stroke models (Woodruff *et al.*, 2011; Lindsey *et al.*, 2018). These models can be broadly categorized into two types: "global" ischemia and "local" or "focal" ischemia. Global ischemia models are generated by occluding two vertebral arteries, two carotid arteries, or all four vessels simultaneously for 5–15 min. These conditions mimic scenarios such as cardiac arrest and coronary occlusion in humans (Tajiri *et al.*, 2013). Focal ischemia models are more relevant to human ischemic events. *In vivo* experiments using the four-vessel occlusion (4-VO) model are commonly performed in rats, mice, dogs, and pigs. This model offers advantages such as ease of preparation, high reproducibility, and low incidence of seizures. Despite their benefits, 4-VO models have certain limitations, including the requirement for a two-step surgical procedure, permanent vertebral artery occlusion, and higher mortality rates. The two-vessel occlusion (2-VO) model is commonly performed in cats, dogs, sheep, and rabbits and involves a single surgical procedure, manageable recirculation, and lower mortality rates. However, the 2-VO model exhibits poor reproducibility and strain-dependent variability (Chung *et al.*, 2014; Popa-Wagner *et al.*, 2014).

Middle cerebral artery occlusion (MCAO) models have been extensively developed for rodents. In aged rodents, MCAO can be induced using either permanent or transient occlusion, typically lasting 30–120 min. Ischemic stroke research uses various MCAO models, classified into seven main types: (i) non-local photothrombotic occlusion by irradiation of the right carotid artery; (ii) thermocoagulation following micro-craniotomy; (iii) intraluminal occlusion using a silicone-coated suture; (iv) mechanical occlusion with hooks attached to a micromanipulator; (v) cauterization; (vi) photothrombotic occlusion; and (vii) thrombus

injection into the external carotid artery or injection of endothelin-1 (Chung *et al.*, 2014; Popa-Wagner *et al.*, 2014). Among these models, middle cerebral artery (MCA) occlusion is the most common, accounting for nearly 50% of ischemic stroke cases involving arterial blockages. MCA occlusion can damage the cortex and striatum, but the extent of infarction depends on several factors, including the occlusion site, duration, and collateral blood flow present in the MCA (Zeng *et al.*, 2023). The MCAO model—particularly those using endovascular filaments—has become the preferred choice in ischemic stroke research because of its ease of use and high survival rates in experimental animals. It has been widely applied to study ischemic stroke etiopathogenesis and to develop and evaluate novel therapeutic interventions (Li and Zhang, 2021; Zeng *et al.*, 2023). Accordingly, various mediators appear to contribute to improved functional outcomes in preclinical *in vivo* models of ischemic stroke.

In recent preclinical *in vivo* ischemic stroke studies are presented in Table 2, various types of MSCs have shown notable therapeutic potential through mechanisms such as neuroprotection, angiogenesis, and antioxidative defense. For example, human embryonic MSCs (hESC-MSCs) administered intracerebroventricularly in Wistar rats MCAO showed enhanced CD31 expression, indicating increased angiogenesis (Mitaki *et al.*, 2020). Similarly, human umbilical cord MSCs (hUCMSCs) administered intravenously in MCAO rats significantly affected neurotrophic aspects, such as BDNF, VEGF, and SDF-1, over 28 days (Yang *et al.*, 2024). In contrast, bone marrow-derived MSCs increased glutathione synthesis, providing antioxidative effects within 24 hours (Yabuno *et al.*, 2023). Additionally, placenta-derived MSCs demonstrated neuroprotection via the ACE-2/Ang 1-7/MasR pathway in mice (Tan *et al.*, 2015). Furthermore, genetically modified BMSCs delivered intracardially to a focal cerebral infarction model improved CXCR4+ cell recruitment (Salikhova *et al.*, 2021).

Human MSCs in preclinical stroke models demonstrate multiple therapeutic mechanisms that contribute to recovery. MSC aggregates (hMSC-agg) reduce ischemic damage through activation of the PI3K/Akt pathway (Kawauchi *et al.*, 2022). Interferon-γ-activated MSCs (aMSCγ) upregulate collagen α-2(VI), aiding tissue remodeling (Hu *et al.*, 2022), whereas intra-arterial bone marrow MSCs elevate G-CSF, IL-10, and VEGF levels, supporting recovery (Hu *et al.*, 2022). Other MSC types—such as B10 and human umbilical MSCs—improve neuroprotection and angiogenesis by increasing neurotrophic factors such as BDNF and VEGF (Namestnikova *et al.*, 2023). Additional therapies—including modified stromal cells and exosome treatments—enhance angiogenesis, reduce apoptosis, and leverage signaling pathways for neuroprotection (Chen *et al.*, 2023, 2024). Additionally, hBMSCs (SB623), when administered intracerebrally,

Table 2. An overview of the effectiveness of MSC therapies in preclinical *in vivo* models of cerebral ischemia along with an emphasis on the possible roles of secretome components.

MSC Therapy	Stroke Model	Route	Dose	Timing post-Stroke	Marker Analysis	Potential role of the secretome	Ref.
Human embryonic MSCs (hESC-MSC)	Adult male Wistar rats (MCAO, 90 min)	ICV	Single dose of CM	7 days	CD31	Angiogenesis	(Asgari Taei *et al.*, 2021)
					Nestin, Ki-67, and DCX	Neurogenesis (differentiation)	
Human umbilical cord mesenchymal stem cells (hUCMSCs)	Rats MCAO over 2 hours	IV	2×10^7 cells/kg	28 days	SDF-1α, CXCR4, Nestin, NeuN	Neurogenesis (migration and differentiation)	(Shen *et al.*, 2023)
					BDNF	Neuroprotection (inflammation)	
					IL-10		
					VEGF-A	Angiogenesis	
BMSCs	Adult male Sprague Dawley rats MCAO for 2 h	IV	1×10^6 cells	24 hour	5-oxoproline GSH (glutathione)	Neuroprotection (oxidative stress)	(Lan *et al.*, 2022)

(Continued)

MSC Therapy	Stroke Model	Route	Dose	Timing post-Stroke	Marker Analysis	Potential role of the secretome	Ref.
Mesenchymal stem cells from human placenta (hPMSC)	Male mice MCAO 1 hour/ Reperfusion 24 h	IP	5 x 10^5 cells	7 days	ACE-2/MasR	Neuroprotection (inflammation)	(Barzegar *et al.*, 2021)
BM-MSCs with LV p38 shRNA	Mouse focal cerebral infarction was treated with photochemical treatment for 24 h	IC	10^6 cells	24 hour	Annexin V-APC/PI CXCR4	Neuroprotection (apoptosis) Neurogenesis (migration)	(Bai *et al.*, 2023)
Human mesenchymal stem cell aggregates (hMSC-agg)	Adult male Sprague–Dawley rats (tMCAO) for 1.5 h	ICV	1.80 µg/ml	7 days	GFAP, DCX NeuN	Neurogenesis (migration, differentiation)	(Helsper *et al.*, 2022)
Female rats interferon-γ–activated mesenchymal stem cells (aMSCγ)	Female Sprague–Dawley rats MCAO 90 min/ reperfusion 3 h	IV	5×10^6 cells/kg	21 days	Collagen α-2(VI), one of the three α-chains of type VI collagen	Neuroprotection (inflammation)	(Tobin *et al.*, 2020)
Human bone marrow-derived MSCs (hBMSCs)	Male MCAO mice for 3 h	IA	9.1 x 10^4 cells	14 days	VEGF, miR-17-92 IL-1α	Angiogenesis Neuroprotection (inflammation)	(Wong *et al.*, 2023)
B10 hMSCs	Adult male Wister rats 90 min MCAO/ reperfusion 2 hours	IC	1 × 10^6 cells/5 µl	24 hour			(Mitaki *et al.*, 2020)
hUMSCs in Wharton's jelly	SD rats (MCAO, 90 min)	Intracerebral	5 × 10^5 cells	56 days	Angiopoietin-2, VEGF, bFGF, PDGF-AA, VEGFR-3 BDNF, bFGF, PDGF-AA	Angiogenesis Neuroprotection (inflammation)	(Fu *et al.*, 2022)
hUC-MSCs	Male murine model of MCAO 90 min	IV	5 × 10^5 cells	28 days	IL-1Ra, IL-4, IL-10, TGF-β, BDNF	Neuroprotection (inflammation and differentiation)	(Yang *et al.*, 2024)
Human bone marrow-derived stromal cells (SB623)	Adult male Sprague Dawley rats MCAO 90 min	Intracerebral	4.0×10^5 cells/5 µl	14 days	VEGF BDNF	Angiogenesis Neuroprotection (differentiation)	(Yabuno *et al.*, 2023)
Autologous bone marrow stromal cell (BMSC)	Rat lacunar infarction	IC	5 × 10^5 cells	6 weeks			(Tan *et al.*, 2015)

(Continued)

MSC Therapy	Stroke Model	Route	Dose	Timing post-Stroke	Marker Analysis	Potential role of the secretome	Ref.
hiPSCs can be derived from human dermal fibroblasts (HDFs)	Adult male Sprague Dawley rats 90 min MCAO	IA	50 µg/ml	30 days	BDNF, GDNF, CNTF, and NGF IL-4, IL-10, and IL-13 Bax/Bcl-2	Neurogenesis (differentiation) Neuroprotection (inflammation and apoptosis)	(Salikhova *et al.*, 2021)
hBMSCs were transfected with a vector encoding the human NICD	Adult male Wistar rats treated for 24 h with tMCAO	IC	4.0×10^5 cells/5 µl	15 days	MCP-1 FGF-1 and FGF-2 BrdU/doublecortin (Dcx)	Neuroprotection (inflammation and apoptosis) Neurogenesis (differentiation)	(Kawauchi *et al.*, 2022)
BMSC-Exos	Male Sprague-Dawley rat MCAO for 60 min	IV	25-50 µg/100 µL	14 days	VEGF, VEGFR2, Ang-1, and Tie-2 miR-21-5p and miR-22-3p	Angiogenesis Neuroprotection (inflammation)	(Hu *et al.*, 2022)
BMSCs differentiate into MSC-NTF cells	Adult male Sprague-Dawley rats (90-min MCAO).	IC	4×10^5 cells/4 µl	6 weeks	BDNF, HGF, NGF, GDNF, and NT-3 VEGF	Neuroprotection (inflammation) Angiogenesis	(Jiang *et al.*, 2022)
hPMSCs	Male Wistar rats (MCAO, 90 min)	intracerebral	3×10^5 cells/15 µl	14 days			(Namestnikova *et al.*, 2023)
Rat BMSCs	Adult female Sprague–Dawley rats MCAO 90 min/ reperfusion for 6 h	IA	$1 * 10^5$ cells	24 hour	SIRT1, NF-κB BDNF	Neuroprotection (inflammation and differentiation)	(Sarmah *et al.*, 2022)
Adipose-derived stem cells (ASCs)	Adult male ICR mice transient (tMCAO) for 1.5 h	IC	3×10^5 cells/10 µL	35 days	CXCR4 and CXCL12 VEGF, bFGF	Neurogenesis (migration) angiogenesis	(Zheng *et al.*, 2024)
Mice BMSCs- EXO	Male mice, a photothrombotic model	IV	100 µg/200 µL PBS	14 days	DCX- and Ki67 BDNF	Neurogenesis (differentiation) Neuroprotection (inflammation)	(R. Xu *et al.*, 2020)
Male GFP-TG BMSCs	Adult male Sprague–Dawley rats, MCAO, 2 h	IV	3×10^6 cells/ml	24 hour	TUJ-1/MAP2	Neurogenesis (differentiation)	(Q. Wang *et al.*, 2022)

(Continued)

MSC Therapy	Stroke Model	Route	Dose	Timing post-Stroke	Marker Analysis	Potential role of the secretome	Ref.
hESC-MSC	Adult male Wistar rats (MCAO, 90 min)	ICV	-	7 days			(Asgari Taei *et al.*, 2021)
Rat BMSC-IONPs (iron oxide nanoparticles)	Male rats MCAO 90 min	IC	10^6 cells/10ml	14 days	IGF-1, HGF, and GDF-15 IGFBP-5	Neuroprotection (inflammation)	(Wu *et al.*, 2020)
Rat BMSCs	Male SD (Sprague Dawley) rats 2 h of MCAO	IC	2×10^5 cells/10 µl	14 days	LC3 GATA 3 Annexin V-FITC VEGF BDNF	Autophagy Neuroprotection (inflammation) Neuroprotection (apoptosis) Angiogenesis Neuroprotection (inflammation)	(Li, Zhong, *et al.*, 2021)
Human BMCs	Adult Sprague Dawley rats MCAO 2 h	IV	$1,2,5 \times 10^6$ cells/ml	28 days			(Y. Chen *et al.*, 2023)
Rat BMCs	Adult male Sprague Dawley rats permanent MCAO	peri-infarction area	5×10^5 cells/4 µl of DMEM medium	28 days	Bax and Bcl-2 Beclin-1 and LC3BII/LC3BI TLR4/NF-κB, IGF-1 VEGF	Neuroprotection (apoptosis) Neuroprotection (autophagy) Neuroprotection (inflammation) Angiogenesis	(J. Chen *et al.*, 2022)
Rat BMCs	Male Wistar rats, MCAO, 3 h	IV and IP	100 µg/kg	23 days	GSH, SOD, and GSH-Px MDA GFAP, CD11b Bax, Bcl2, and Bad Synaptophysin (SYN)	Neuroprotection (antioxidant) Neuroprotection (oxidative stress) Neuroprotection (inflammation) Neuroprotection (apoptosis) Neurogenesis (differentiation)	(Dhir *et al.*, 2023)

(Continued)

MSC Therapy	Stroke Model	Route	Dose	Timing post-Stroke	Marker Analysis	Potential role of the secretome	Ref.
Rat BMSCs	Male Wistar rats lacunar infarct 5 min	IC	5×10^5 cells/10 µl	49 days	NeuN, DCX GFAP BDNF HGF NGF	Neurogenesis (differentiation) Neuroprotection (inflammation) Neuroprotection (inflammation) Neuroprotection (oxidative stress) Neurogenesis (differentiation)	(Shichinohe *et al.*, 2013)
hPMSCs	Male Wistar rats (MCAO, 90 min)	IA	5×10^5 cells/2 ml	24 h			(Gubskiy *et al.*, 2021)
Rat BMSC-Exos with Musk Ketone	Male SD rats, MCAO 2 h/ reperfusion for 24 h	IV	2 mg/kg of Exo	24 hour	Bcl-2 *Bax*, cleaved caspase 3 IL-6 and COX-2.	Neuroprotection (apoptosis) Neuroprotection (inflammation)	(C. Chen *et al.*, 2024)
Human BMSCs	Adult nestin–GFP–TG mice MCAO	Transcranial injection into the periischemic area	1.0×10^5 cells/µL	15 week	Sox2, Nestin, Tuj1, NCAM1, and Ki67 MGs/MΦs, TNF alpha, NF-κB	Neurogenesis (differentiation) Neuroprotection (inflammation)	(Fujiwara *et al.*, 2024)
Human BMSC-IONPs (iron oxide nanoparticles)	SD rats male 60 min MCAO	IV	200 µg/300 µl	24 hour	Inos, nNOS, Arg-1, TNF--α, IL-1β, COX-2,GFAP MAP2	Neuroprotection (inflammation) Neurogenesis (differentiation)	(Kim *et al.*, 2020)
BMSC-EVs	SD male rats with permanent MCAOs	IV	1×10^{10}cells	24 hour	Caveolin-1 Claudin-5	Angiogenesis Angiogenesis	(Li, Liu, *et al.*, 2023)
hUC-MSC	Adult male mice (90 min of MCAO)	IV	10^6 cells	21 days	Annexin V-FITC	Apoptosis	(Li, Huang, *et al.*, 2023)
BMSC-Exos; miR-193b-5p Exos	Male Sprague Dawley rats MCAO 4 h/24 h reperfusion	IV	200-mg exosomes	3 days	miRNA-193b-5p, AIM2, GSDMD-N, Cleaved Caspase-1, IL-1b/IL-18	Neuroprotection (inflammation)	(Y. Wang *et al.*, 2023)

(Continued)

MSC Therapy	Stroke Model	Route	Dose	Timing post-Stroke	Marker Analysis	Potential role of the secretome	Ref.
Mice BMSCs-Exos	Adult mice mice MCAO/1 h reperfusion	IV	100 μg /100 μL	3 days	Bax, Bcl-2, Annexin V-FITC MDA GSH-Px KLF4 (Kruppel-like factor 4)	Neuroprotection (apoptosis) Neuroprotection (oxidative Stress) Neuroprotection (Antioxidant) Neuroprotection (inflammation)	(Q. SouthS. Wang *et al.*, 2023)
hUCMSCs	MCAO/reperfusion 2hours/45min	IV	200 μg	7 days	TLR4/NF-κB and miR-125b-5p	Neuroprotection (inflammation)	(Qiu *et al.*, 2022)
hPMSCs	Male Sprague-Dawley rats (MCAO) in 120 min	IV	1×10^6 cells/200 μl	16 days	CXCL12/CXCR4	Neurogenesis (migration)	(Li *et al.*, 2021)
3D spheroids of UCMSCs	Female nude mice (MCAO)	IC	5×10^5 cells	14 days	BDNF, IGF-1 VEGF	Neuroprotection (inflammation) Angiogenesis	(Hsu *et al.*, 2021)
hADSCs	Male SD rats MCAO of 3 days	ICV	1×10^{10} particles/ml	3 days	Bax/Bcl-2 miR-146a/miR21	Apoptosis Neuroprotection (inflammation)	(Jang *et al.*, 2024)
hUMSC-Exos	MCAO murine model of ischemic stroke, 60 minutes	IV	50 μg/250 μl	72 hour	iR-146a/miR21, IRAK1/TRAF6, IL-6, TNF-α, IL-1β, NFκB, Toll-like receptor (TLR)	Neuroprotection (inflammation)	(Z. Zhang *et al.*, 2021)
iMSC-sEV	Male Sprague Dawley rat MCAO for 2 hours	IV	1×10^{11} cells/500 μl	28 days	STAT3 LC3-II/LC3-I, Beclin-1, P62	Angiogenesis Neuroprotection (autophagy)	(Xia *et al.*, 2020)
hNSCs	Male Sprague Dawley rats MCAO 1h	IC	2 μl	24 hour	PRDX6	Neuroprotection (Antioxidant)	(Tang *et al.*, 2021)

elevated VEGF and BDNF levels, promoting recovery (R. Xu *et al.* (2020). Other approaches—such as administering autologous BMSCs into models of lacunar infarction—showed beneficial effects, particularly related to insulin-like growth factor 1 (Wang *et al.*, 2022).

Furthermore, MSCs derived from human adipose tissue, administered intracranially, increased miR-146a and miR-21 expression, contributing to neuroprotection (Hsu *et al.*, 2021). Studies utilizing three-dimensional spheroids of MSCs in MCAO models revealed the capacity to deliver paracrine factors, such as BDNF and IGF-1, further supporting recovery mechanisms (Li *et al.*, 2021). In summary, these results emphasize the varied therapeutic potential of MSCs and their derivatives in promoting stroke recovery while underscoring the need to improve delivery techniques and gain a deeper understanding of the underlying mechanisms for future clinical use.

Conclusion

The use of MSCs and their secretomes represents a groundbreaking approach to managing ischemic stroke, effectively addressing the limitations of traditional therapies. Preclinical research has demonstrated that MSCs and their secretomes offer substantial neuroprotective, anti-inflammatory, and angiogenic benefits, significantly supporting functional recovery in ischemic stroke models. MSC-secretome-based therapies, particularly those involving extracellular vesicles and exosomes, have yielded promising results in enhancing neuronal survival and facilitating vascular repair. These effects are primarily mediated by the regulation of inflammation and stimulation of neurogenesis pathways. *In vitro* and *in vivo* models provide essential insights into the interaction of secretomes with diverse cell types implicated in ischemic stroke pathology. Despite these promising findings, the translation of MSC therapies into clinical practice requires further refinement, particularly in terms of delivery routes, dosing protocols, and preconditioning strategies. Addressing these translational challenges could facilitate the integration of MSC-based therapies into standard clinical practice for stroke, providing a safer and more versatile treatment option to enhance recovery and improve patient outcomes.

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Gita Serafika Shannon drafted the manuscript. Ratih Rinendyaputri and Amarila Malik revised and edited the manuscript. Ratih Rinendyaputri, Amarila Malik, and Sunarno critically checked the manuscript. Gita Serafika Shannon, Ratih Rinendyaputri, and Amarila Malik edited the manuscript. All authors have read and approved the final version of the manuscript.

Data availability

All references are open-access, so data can be obtained from the online literature.

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