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REVIEW

In vitro and In vivo Studies on Mesenchymal Stem Cells for Ischemic Stroke Therapy: A Scoping Review of The Therapeutic Effect

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Introduction: Mesenchymal stem cells (MSCs) have a paracrine impact and may regenerate a variety of tissues. This represents a new prospect in cell-based stroke treatment. Several in vitro and in vivo investigations have demonstrated the neuroprotective and neurogenesis properties of MSCs and their secretome.

Purpose: This review provides a comprehensive analysis of the therapeutic effects of MSCs and their secretome on stroke models in vitro and in vivo.

Methods: A coverage evaluation is undertaken in accordance with PRISMA-ScR principles. The selection procedure includes the identification of items. Scopus site, PubMed and ScienceDirect, are used for in vitro and in vitro research, including electronic searches. The search terms include "ischemic stroke" or "MCAO", "MSC", "secretome", and "neurogenesis" or "angiogenesis". The searches are limited to English-language articles with full text availability.

Results: After selecting 390 papers from two search engines, 94 publications satisfied the review criteria for using MSCs and secretomes for ischemic stroke treatment. We comprehensively review both in vitro and in vivo studies, analyzing aspects such as the source and treatment of MSCs and secretomes, as well as administration, dosage, and mechanisms of therapeutic effects in stroke models.

Conclusion: MSC and secretome therapy for stroke have shown promising results in both in vitro and in vivo models. Exploration of alternative MSC sources, refining of isolation techniques, transfection of various proteins, and combination with herbal medicine are all efforts to improve the preclinical model. This work can be used as a reference for preclinical researchers to help with research design and translational research in clinical trials.

Keywords: middle cerebral artery occlusion (MCAO), conditioned medium/CM, secretome, mesenchymal stem cells (MSC), stroke ischemic

Introduction

Mesenchymal stem cells (MSCs) can be obtained from various sources such as adipose tissue, umbilical cord, bone marrow, iPSC-MSC and peripheral blood.[1–7] Bioactives secreted by MSCs have a paracrine effect because they contain various proteins that play a role in the neurogenesis process such as brain derived neurotrophic factors/BDNF, neurotrophic growth factor/NGF, and stromal derived factor-1/SDF-1.[8–10] The angiogenesis process in the penumbral area also helps the neurogenesis process so that vascular endothelial growth factor/VEGF is urgently needed, which can be supplied by MSC secretions.[11] The effects of neuroprotection through inflammatory pathways, apoptosis, and autophagy are also important effects to continue researching.[12,13] The role of EVs, exosomes and microRNAs contained

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in MSC secretome/conditioned medium (CM) is a factor in the effectiveness of therapy considering that it is a regulator of various genes.[14,15]

Mesenchymal stem cells and their secretomes provide paracrine effects, especially for ischemic stroke.[16] MSCs play a role in the regeneration of the blood-brain barrier (BBB) linkage in brain tissue, which can suppress inflammation so that the neuroregeneration and neuroprotection processes can take place properly.[17,18] The bioactive role of MSC secretomes and methods to increase their potential in angiogenesis, neurogenesis, and neuroprotection in ischemic stroke therapy continue to be carried out in vivo and in vitro.[19–21] This is because, while experimental investigations have been successful, systematic review studies looking at clinical trials of MSC treatment in stroke patients have not yielded meaningful benefits. Several systematic reviews of clinical trials found that MSC therapy did not result in substantial improvements in ischemic stroke patients. The heterogeneity of data, including MSC sources, doses, replications, and delivery, as well as patient severity, all contributed to variances in therapeutic success.[22–24]

Currently, there is a gap in translational research from preclinical trials to clinical trials that must be bridged to address clinical trial issues. This study will examine the literature on the effects of MSC and secretome therapy in vitro and in vivo, numerous efforts to optimize MSC and its secretome to have an impact on the therapeutic effect, as well as its potential pharmacological use in ischemic stroke models. This study is planned to serve as a reference for researchers conducting preclinical trials to support the success of subsequent clinical trials.

Materials and Methods

We conducted this scoping review according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) statement and based on the Joanna Briggs Institute/JBBI guidelines.[25,26] We formulated the research objectives and questions by referring to the Problem, Concept and Contest (PPC), as problem: model stroke ischemic. Concept: MSC and MSC secretomes for stroke ischemic therapy, and contest: in vitro and in vivo study. The research question was "What types of MSCs and their secretomes have been used for ischemic stroke therapy in preclinical trials?". The aim of this review was to provide a comprehensive analysis of the therapeutic effects of MSCs and their secretomes on stroke models in vitro and in vivo.

Search Strategy

The article searches strategy takes a thorough approach to finding relevant research articles. Advanced search techniques, including "AND" and "OR" operators, are employed to filter results. The search by PICO is ((((((Ischemic Stroke[MeSH Terms]) OR (stroke[Title/Abstract])) OR (brain ischemic[Title/Abstract])) OR (Middle Cerebral Artery Occlusion[Title/Abstract])) OR (MCAO[Title/Abstract])) AND ((((((((mesenchymal stem cells[MeSH Terms]) OR (mesenchymal stem cell[Title/Abstract])) OR (MSC[Title/Abstract])) OR (secretome[Title/Abstract])) OR (Conditioned medium[Title/Abstract])) OR (Exosomes[Title/Abstract])) OR (Extracellular vesicles[Title/Abstract])) OR (EVs[Title/Abstract]))) AND ((((neuroprotection[MeSH Terms]) OR (angiogenesis[Title/Abstract])) OR (autophagy[Title/Abstract])) OR (neurogenesis[Title/Abstract])). The search focuses on electronic resources like ScienceDirect and PubMed.

Study Selection

Inclusion criteria for this scoping review include in vitro and in vivo research articles, with ischemic stroke models using MSC and/or with MSC secretome in English, last 10 years, and full English text availability. Based on these inclusion criteria, clinical trial research studies with only abstracts are available.

Data Extraction

Two independent reviewers carefully choose articles and extract data based on inclusion criteria to ensure the scoping review is complete and accurate. The table summarizes the evaluation results for further analysis. Journal articles are obtained using both electronic and manual searches. The abstract is initially picked based on its relevance to the study topic. The second stage comprises additional selection based on the article's substance and adherence to inclusion and exclusion criteria. Finally, data from qualifying articles is extracted and processed for analysis.

Results

Study Inclusion

The PRISMA diagram (Figure 1) illustrates the systematic selection process of articles for a scoping review. Originally, a total of 390 articles were obtained from two search engines: PubMed and ScienceDirect. After undergoing screening and selection based on inclusion and exclusion criteria, as many as 134 articles considered suitable for review. Furthermore, 134 selected articles were thoroughly read and analyzed for extraction relevant data. Characteristics of inclusion were compiled and presented in Tables 1–4.

Characteristic of Included Studies

These articles include in vivo test articles, in vitro test studies, and both. In the in vivo test describe allogenic approaches that involve bone marrow-derived MSCs. Meanwhile, the remaining articles used human MSCs (xenografts), primarily using mice as stroke model animals. Most researchers used allogeneic MSCs from adipose and bone marrow, whereas xenografts used human MSCs from umbilical cord and fat tissue. In stroke models, most transplants deliver MSCs, but some also deliver CM (six article) and exosomes/EVs (26 article). The use of MSCs for stroke therapy has been modified in many ways, including hypoxia, gene transfection, and the addition of herbal medicine.[80]

The in vivo test employs the middle cerebral artery occlusion (MCAO) stroke model, whereas the in vitro test uses the oxygen glucose deprivation (OGD) approach. For in vitro studies, the researchers used cell lines as well as primary cultures from mouse brain tissue. In the in vivo test, various concentrations were delivered intravenously, intra-arterially,

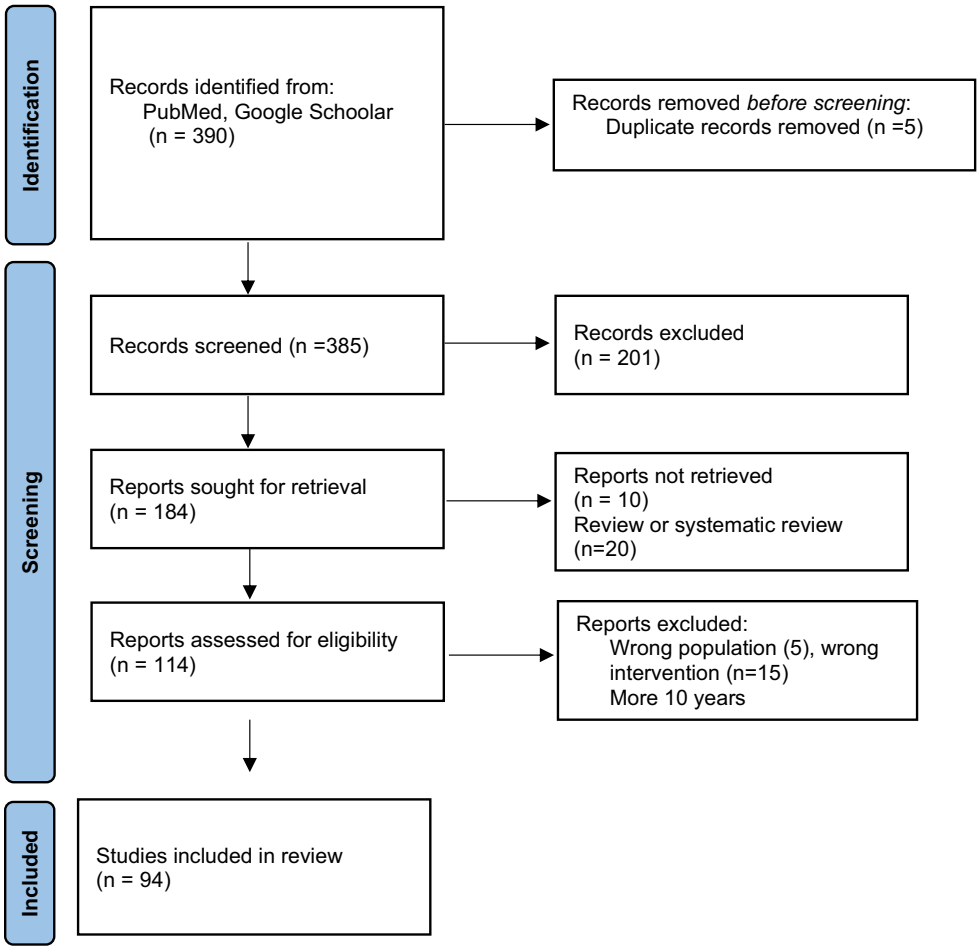

Figure 1 The study selection flow chart. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses Scoping Review (PRISMA-ScR) flow chart depicts the amount of data identified, included, and eliminated during the various rounds of a systematic review.

Table 1 Characteristic of Included Studies (Administration via Intra Artery)

No	Author (Year)	Study Design	Population			Intervention	Outcome	
			Animal/Cell	Method		Dose	Marker	Final Effect
1.	Otsu et al, (2023)[1]	In vivo	Rat	Ischemic	Xenograft	HuPBMC -hypoxia (1.10^6/0,5µL via IA)	miR-155, HIF-1α, VEGF, TGF-β1, TGF-β2, IL-1β, TNF-1α	Neurogenesis and angiogenesis
		In vitro	Primary mouse microglia	OGD				
2.	Liu et al, (2019)[27]	In vivo	Rat	MCAO	Allognic	Rat BMMSC transfected mitochondria. (0.5 × 10^6/ 10µL MSCs via common carotid artery)	TTC, Annexin V, DsRed2 +/Hoechst 33342,	Angiogenesis
3.	Oh et al, (2015)[28]	In vivo	Rat	MCAO	Xenograft	Human AD-MSC (5x 10^5 /5 mL via IA)	hNu, Nestin, MAP2, NeuN, GFAP, DCX, ED1, Behavior test, VEGF, BDNF,Nestin, type III btubulin	Neuroprotective

intranasally, and intracerebral. Similarly, in vitro testing of therapeutic models using co-culture or growing secretome or EVs at specific doses. The researchers discovered that using MSCs as well as EVs or secretions resulted in angiogenesis, neurogenesis, and neuroprotection. This neuroprotection is demonstrated by the influence on anti-inflammatory, anti-apoptosis, and haemostasis processes via autophagy. Only modest neuroprotective effects, particularly in autophagy, were seen in this investigation (9 studies).

Neuroprotection, Angiogenesis, and Neurogenesis Effect of Therapy

This study used a scoping review to determine the administration method, dose, and therapeutic effects of MSC-CM for stroke therapy. This investigation revealed that there are still a few research reports on intra-arterial delivery, with three articles reporting from various sources of MSC and doses. The dose was $5x10^5$-$1x10^6$, and the sources were PBMC, adipose tissue derived mesenchymal stem cells/AD-MSC, and bone marrow derived mesenchymal stem cells/BMMSC from humans and rats. For in vivo testing, use the MCAO rodent model. According to the current review, MSC have been shown in vitro to reduce inflammation, and in vivo, their therapeutic effect can decrease apoptosis, increase angiogenesis, and neurogenesis (Table 1).

Table 2 shows that intravenous injection was more frequently reported in the MCAO model, both transient and permanent. The stroke model was tested in vitro using the oxygen glucose deprivation/OGD approach. Lipopolysaccharide/LPS induction was employed to produce an inflammatory response. Therapies using MSC, MSC-CM and extracellular vesicles/EVs including exosomes at various doses have been reported with intravenous administration.

Intranasal treatment has also been used in stroke models with MCAO in rats and mice. However, analogous research demonstrating therapeutic efficacy in stroke models is still rare. The various cell sources make it challenging to choose the optimum cell type for intranasal MSC therapy (Table 3). Stroke therapy through intracranial has been reported. The use of in vitro models with cell lines such as SH-SY5Y and BV2 can be used to see the effects of neuroinflammation, neuroprotection through apoptotic and autophagy pathways (Table 4).

Mesenchymal stem cell/ MSC treatment and secretome have a neuroprotective impact through paracrine actions, which helps to decrease inflammation and apoptosis while promoting autophagy.[29,40,82] In addition to the up and down regulation of gene expression and protein markers, TTC is used to measure the infarct area. Several studies have found that therapy resulted in a reduced infarct area than sham control, indicating a neuroprotective effect.[40,45]

Neurogenesis and angiogenesis are characterized by several markers of neuronal differentiation and blood vessel development.[83–85] In in vitro, it may be demonstrated with HUVEC cells and neural progenitors, as well as other and primary cells.[36,50] In in vivo mouse experiments, post-therapy analysis was performed by examining the increase in neuronal differentiation and blood vessel creation using the Y maze, Morris water, and rotarod.[40,75,76]

Table 2 Characteristic of Included Studies (Administration via Intravenous)

No	Author (Year)	Study Design	Population			Intervention	Outcome	
			Animal/Cell	Method		Dose	Measure/Marker	Final Effect
1.	Kuang et al, (2020)[29]	In vivo	Mice	MCAO 12 hrs	Allogenic	miR-125/Evs from mice ADMSC ($2x10^3$ −10^5 via femoral vein)	p53, LC3-I, LC3-II, NeuN, MTT, TTC	Neuroprotection
		In vitro	Fetus mice -primary cortical neuron	OGD 4, 8, 10 hrs		Co-culture ADMSC 24 hrs		
2.	You et al, (2023)[28]	In vivo	Mice	MCAO 1,5 hrs	Xenograft	hMSC-derived Apoptotic vesicles (1.6 mg/kg via IV)	iNOS, Agr1, TNF-α, IL-1β, IL-4. IL-6, IL-10, TGF-β, PC-12, Bcl2/Bax, MAP, MMP-9,	Anti inflammatory
		In vitro	PC 12 cell, BV2 microglia, BCECs	OGD 2 hrs, LPS, Scratch assay dan matrigel tube formation				
3.	Gregorius et al, (2021)[30]	In vivo	Mice	MCAO	Xenograft	hBMMSC-EVs normoxia and hypoxia ($2x10^6$/200µL via IV)	miR-126-3p, miR-140-5p, let-7c-5p, miR-186-5p, miR-370-3p, miR-409-3p, tube formation, migration assay, MTT	Angiogenesis
		In vitro	BCECs/hCMEC/D3	Transwell Migration Assay, Tube Formation Assay				
4.	Son et al, (2023)[31]	In vivo	Rat	tMCAO	Xenograft	3D hWJ-MSC-EVS ($3x10^7$-10^{10} EVs/rat via IV)	Histone H2A.Z, histone H3, lamin A/C, miR-27a-3p and miR-132-3p, Ki67, MRI	Neurogenesis
5.	Han et al, (2023)[32]	In vivo	Mice	MCAO	Allogenic	3D and 2D BMMSC-Exo (100µg/100µL via IV)	TNF-α, IL-6, IL-10, CD31, TGF-β1, TCC	Neuroprotection and angiogenesis
		in vitro	Cell line BV2	LPS				
6.	Yang et al, (2017)[15]	In vivo	Mice	Focal cortical ischemic	Allogenic	Mice BMMSC-Exo/miR-124 (12 mg via IV)	Sox2, Hoechst, Nestin, DCX,	Neurogenesis
7.	Lu et al, (2023)[5]	In vivo	Mice	MCAO	Xenograft	hiPSC-MSC-Exo (100 µg Exo via IV)	VEGF, CXCR4, Calnexin, Tubulin, SDF-1	Angiogenesis
		In vitro	HT-22 murine hippocampal	OGD 2 hrs		hiPS-MSC-EV (100 µg/mL)		
8.	Tang et al, (2022)[33]	In vivo	Rat	MCAO	Allogenic	BMMSC tibia femur + stroke serum ($2x10^6$ via IV)	TTC, Tunnel, BDNF, NeuN, DCX, Brdu, HGF, NGF, VEGF, IL-1β, IL-6, TNF-α	Neuroprotective
9.	Zhang et al, (2023)[34]	In vivo	Mice aged	MCAO	Xenograft	hBMMSC ($1x10^6$ via vein tail)	CD31, Brdu, MAP2, Brdu, rotarod, water maze	Angiogenesis
10.	Moon et al, (2018)[35]	In vivo	Rat	tMACO	Allogenic and xenograft	Rat and human BMMSC The rMSCEVs or fibro-EVs (30 µg/rat) or hMSCs (1–2 × 10^6 cells via IV)	VEGF, HIF-1alfa, TGFbeta, PDGF, Angiopoetin1,	Angiogenesis
		In vitro	HUVEC	Tube formation assay				
11.	Sheikh et al, (2019)[36]	In vivo	Rat	MCAO	Xenograft	Silencing IL-1β in B10 cell (clone of immortalized bone marrow cells). ($3x10^6$/100µL cell via the jugular vein)	IL-1β, VEGF, TGFβ, NeuN, Angiogenin1/2, PDGF, TNF-α	Angiogenesis
		In Vitro	A human microglia cell line (HMO6)	OGD 4 hrs				

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Table 2 (Continued).

No	Author (Year)	Study Design	Population			Intervention	Outcome	
			Animal/Cell	Method		Dose	Measure/Marker	Final Effect
12.	Mu et al, (2019)[6]	In vivo	Rat	MCAO	Xenograft	hATMSC (2x10^6/mL via tail vein)	TTC, GFAP, RECA-1, Rogers'Test, Cylinder Test, StickyLabelTest	Angiogenesis
13.	Zhang et al, (2023)[37]	In vivo	Rat	MCAO	Xenograft	VCAM-1+ hUC-MSCs (1x10^6 via a tail vein)	CCK-8, VCAM-1, NLRP3, Caspase1, TNF-α, IL-6, IL-1β, and IL-18	Neuroprotection and angiogenesis
		In vitro	Cell line SH-SY5Y	OGD				
14.	Wang et al, (2022)[38]	In Vivo	Mice	MCAO	Xenograft	hBMMSC-Evs or MSC-sEVs (2×10^6/200μL via IV)	TUNEL+ cells, NeuN, CD45, CD31, leukocytes, Ly6G+PMNs, and ICAM-1	Neuroprotection
		In Vivo	Mice	MCAO		Human urine-derived stem cells/USC-Exos (1x10^{11} via IV)		
15.	Lee et al, (2020)[39]	In Vivo	Rat	MCAO	Xenograft	HuUC-MSC + transfected CCL2 (1 × 10^6/0.5 mL via tail vein)	TTC, Cresyl Violet, CCL2, CCR2, NeuN, BrdU, RECA, VEGF, GFAP, Iba-1, ED1, iNOS, CD206, and DCX	Neurogenesis, angiogenesis
16.	Zhou et al, (2021)[40]	In Vivo	Rat	MCAO	Allogenic	MSC- Salidroside (p-hydroxyphenethyl-β-D-glucoside, Sal) and MSC pretreated with 0.75 μg/mL salidroside) (10 × 10^6 cells/10μL via caudal side of the frontal fontanel)	Behavioral tests, TTC, CCK-8, Annexin V	Neuroprotection
17.	Hu et al, (2022)[41]	In Vivo	Rat	MCAO	Allogenic	Rat BMMSC-Exos (25 μg/100μL PBS via tail vein)	VEGF, VEGFR2, Ang-1, and Tie-2 Neurological function, TTC, VEGF, scratch assay, transwell migration, tube formation	Angiogenesis
		In vitro	HUVEC	Transwell Migration Assay, Tube Formation Assay, Scratch Wound Healing Assay				
18.	Xu et al, (2020)[42]	In Vivo	Mice	tMCAO	Allogenic	Mice ADMSC-Exo (MADMSC transfected CircAkap7 (400 μg of protein via vein tail)	TTC, Lamin1, NRF2, IL-6, Autophagic vacuoles (autophagosomes), and TNF-1α	Neuroprotection
		In Vitro	Primary astrocytes from the cerebral cortex of mice					
19.	Jiang et al, (2019)[2]	In vivo	Rat	MCAO	Allogenic	Rat CM-BMMSC treatment hypoxia and normoxia (250 μL via IV)	GFAP, CD-31, Akt, TTC, NeuN, Caspase-3, Tunnel, PI3K, Akt,	Neuroprotection
20.	Diekhorst et al, (2019)[43]	In vivo	Rat	MCAO	Xenograft	hATMSC (1×10^6/1 mL of 0.9% NaCl via the tail vein)	GFAP, CD-31, DCX,	Angiogenesis
21.	Zhang et al, (2018)[44]	In vivo	Mice	tMCAO	Allogenic	Mice MSC +Borneol (0,5 x 10^6/0.2 mL sterile PBS via caudal vein)	NeuN, GFAP, TUNNEL, neurological deficits	Neuroprotective
22.	Nazarinia et al (2019)[45]	In vivo	Rat	MCAO	Xenograft	CM-hAMMSC (1 mL IV)	TTC, mTOR, LC3-I/II, Nissl, Tunnel assay,	Neuroprotective
23.	Dumbrava et al, (2021)[46]	In vivo	Rat young and aged	MCAO	Xenograft	hBMMSC-Evs (2× 10^6 or 2× 10^7/1 mL of 0.9% NaCl via tail vein)	DCX, CD31, Rotating pole test, Cylinder test, ED1, Iba-1	Angiogenesis
24.	Liu et al, (2022)[47]	In vivo	Rat	MCAO	Allogenic	Rat BMMSC (1×10^6 via the tail vein)	VEGF, GDNF, TTC	Neuroprotective

25.	Xiao et al, (2023)[48]	In vivo	Mice	tMCAO		BMSCs-Exo were transfected with the shRNA against Egr2 (100 μg via the tail vein)	Annexin V, tube formation, Tunel, Calnexin, Bax, Bcl2, VEGF, HES1, eNOS,	Angiogenesis
		In Vitro	N2/mouse neuroblastoma and bEnd.3 cells/ endothelial cell	Tube formation	Allogenic			
26.	Liu et al, (2018)[49]	In vivo	Rat	tMCAO	Allogenic	Rat BMMSC+ Icariin (ICA) (5 × 10^6 MSCs via tail vein and ICA 60 mg/kg/d via intragastric)	BDNF and VEGF, behavior, PI3K, ERK1/2	Neurogenesis and angiogenesis
27.	Jiang et al, (2018)[12]	In vivo	Murine	MCAO	Xenograft	Rat ADSCs-Exos transfected microRNA (miR)-30d-5p (Exos in 0.9% saline 80 μg per 2 mL via IV)	Anti inflammatory cytokines IL-4, IL-10, and miR-30d-5p d, Beclin-1 and Atg5	Neuroprotection
		In vitro	Primary culture microglia from neonatal rats	OGD	Allogenic	Rat ADSC-Exo (10 μg/mL) under OGD conditions for 6 h		
28.	Moon et al, (2018)[50]	In vivo	Rat	tMCAO	Xenogfrat	hMSCs + 10% FBS, 10% normal serum (NS), or 10% patient stroke serum (SS) (2 x 10^6 cells via IV)	VEGF, GDNF, FGF2, b-galactosidase (SA-b-gal), DCX	Neurorestoration
29.	Xia et al, (2020)[7]	In vivo	Rat	MCAO	Xenograft	iMSC-sEV (1 × 10^{11} particles/500 μL PBS via IV)	MAP2, CD31, CD34, migration and tube formation of endothelial cells, Beclin-1, mTOR, p62, LC3, DCX, SVZ, NeuN, Tunel	Angiogenesis
		In vitro	HUVEC	OGD and tube formation assay				
30.	Lin et al, (2017)[51]	In vivo	Rat	MCAO	Xenograft	hUC-MSCs (1–4 x 10^6/mL via IV)	NeuN. DCX, Iba-1, TUNEL	Neurogenesis
31.	Haupt et al, (2020)[52]	In vivo	Mice	MCAO	Allogenic	MSC preconditioning with lithium (MSCLi-EVs) (2 ×10^6/100μL cell IV)	GFAP, NeuN, GDNF, EGF, BDNF, VEGF, TNF-α, CD31, NF-κB, TLR4, iNOS, SOX2, TBARS, Dcx, TUNEL	Neuroprotection
		In vitro	Astrocytes and microglia - new born mice	OGD		EVs (2 × 10^6cell equivalents for each condition or 13.5 μg EV protein)		
32.	Oh et al, (2018)[53]	In vivo	Rat	MCAO	Xenograft	IV-hUMSCs ($1x10^5$ −$1x10^6$/500 μL of saline via IV)	DCX, TGF-β1, VEGF, HGF, IL1RN, IL1B,TNF, IL6, MMP9, IL-4, IL-10, IL-1ra, TUNEL, pCREB, ED-1, Iba-1, iNOS, and CD206	Neuroprotection
33.	Choi et al, (2018)[21]	In vivo	Rat	MCAO	Xenograft	hUCMSC (1 × 10^6 cells/ 0.5 mL PBS via IV)	hNu, CD63, BrdU/DCX, NeuN	Angiogenesis
34.	Yang et al, (2018)[54]	In vitro	Primary rat BMECs were transfected with plasmids encoding miR-181b-5p, miR-212-5p, or TRPM7	4h OGD	Xenograft		HIF-1α, VEGF, TRPM7, VEGF, miR-130a-3p, miR-93-3p, miR-212-5p, miR-20a-5p, miR-181a-5p, miR-181d-5p, tube formation, migration assay	Angiogenesis
35.	Xin et al, (2017)[55]	In vivo	Rat	MCAO	Xenograft	MSC-exosomes (miR-17-92) (100 μg/0.5 μL PBS via IV)	PI3K/Akt/mTOR/GSK-3β	Neuroplasticity
36.	Geng et al, (2019)[56]	In vivo	Rat	MCAO	Xenograft	Human ADSCs (miR-126 or miR-126 inhibitor via IV)	NeuN, Iba-1, Tunel, TNF-α, IL-1β, Caspase-3, miR-126	Neurogenesis
		In vitro	The mouse BV2 microglial cells	OGD for 6 hours		Anti-inflammation		
37.	Nam et al, (2015)[57]	In vivo	Rat	MCAO	Allogenic	hBMMSC (2 × 10^6 via IV)	MMP-2, NeuN, GFAP, Collagen IV	Neurogenesis

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Table 2 (Continued).

No	Author (Year)	Study Design	Population			Intervention	Outcome	
			Animal/Cell	Method		Dose	Measure/Marker	Final Effect
38.	Zhang et al, (2019)[58]	In vivo	Rat	MCAO	Allogenic	Rat BMSCs were transfected by the tsp4 virus/ TSP4-BMSC (2 × 10^6 /mL) via the caudal vein)	Tube formation, TSP4, VEGF, MMP2, MMP9, Ang-1	Angiogenesis
39.	Zhang et al, (2019)[59]	In vivo	Mice	MACAO	Allogenic	Mice BMMSC-RGD-exo (miR-210 via the tail vein)	VEGF, Integrinβ, CD34	Angiogenesis
40.	Sabbaghziarani et al, (2017)[10]	In vivo	Rat	MCAO	Xenograft	WJ-MSCs ± retinoic acid/RA (1 μM) ± triiodothyronine T3 (25 μg/kg) (1 × 10^6 via caudal vein)	RXRβ, BDNF, Sox2, IL-6, TNF-α	Neuroprotective
41.	Zhang et al, (2016)[60]	In vivo	Rat	MCAO	Allogenic	Sodium ferulate (SF) and n-butylidenephthalide (BP) + rat BMSC (2 × 10^6/mL via caudal vein)	AKT, mTOR, VEGF, BDNF, class III β-tubulin (Tuj1)	Angiogenesis
		In vitro	BMMSC +SF and BP	MTT		BMSC, the cells (1 × 10^5 cells/mL), SF (400, 200, 100, 50, 25, 5, 1, 0.1 and 0.01 μg/mL), BP (4, 2, 1, 0.75, 0.5, 0.25, 0.125, 0.01 and 0.001 μg/mL)		
42.	Zhang et al, (2017)[61]	In vivo	Rat	pMCAO	Allogenic	Rat BMMSC+Sodium ferulate (SF) and n-butylidenephthalide (BP), (2x10^6 cells/mL via the caudal vein. SF (60 mg/kg) was intraperitoneally, BP (10 mg/kg) was subcutaneously)	GFAP, VEGF, DAPI, BDNF, AKT, mTOR,	Angiogenesis
		In vitro	Astrocytes derived from the cortex of normal Rattus norvegicus and HUVEC	OGD, Tube formation				
43.	Yang et al, (2015)[8]	In vivo	Rat	MCAO	Allogenic	Rat BMMSC, IV, (5 x 10^6 cells/mL DMEM/F12 medium via tail vein)	VEGF, Ki67, Hoechst 33342elabeled BMSC	Angiogenesis
44.	Chen et al, (2017)[62]	In vivo	Rat	MCAO	Allogenic	Rat BMMSC hypoxia (1% O2 2,4,8,12,24 hr reoxygenasi 24 hr) and normoxia, N-BMSCs (2 x 10^6) and H-BMSCs (2 x 106^6), respectively, via the tail vein)	MTT, BDNF, VEGF, Caspase-3, Nestin, b-Tubulin, GFAP, TTC	Neurogenesis, angiogenesis
45.	Jahromi et al, (2018)[63]	In vivo	Rat	MCAO		Simvastatin and BMSCs, 3 ×10^6/ mL PBS via tail vein) and Simvastatin (40 mg/kg IP)	GFAP. KI67	Neurogenesis
46.	Shen et al, (2016)[64]	In vivo	Rat	MCAO	Allogenic	3-Methyl-1-phenyl-2-pyrazolin-5-one (MC-186) and rat BMMSC (MCI-186 3 mg/kg via IP, BMMSC 2 × 10^6 via the tail vein)	CXCR4, SDF-1	Neurogenesis
47.	Gutiérrez-Fernández et al, (2015)[65]	In vivo	Rat	MCAO	Xeno and allogenic	hAD-MSCs and rAD-MSCs (2 × 10^6 via IV)	VEGF, GFAP, (synaptophysin) SYP	Angiogenesis
48.	Li et al, (2017)[66]	In vivo	Rat	MCAO	Allogenic	Tetramethylpyrazine (TMP) preconditioning could enhance BMSCs (1 × 10^6/mL PBS via the tail vein)	SDF-1 and CXCR4	Angiogenesis
49.	He et al, (2021)[67]	In vivo	Rat	MCAO	Xenograft	Human olfactory mucosa mesenchymal stem cells (h OM-MSC) (5.0 × 10^6 via tail vein)	Caspase3, GOLPH3, SPCA1, LC3I, LC3 II, LAMP1, Akt, mTOR, PEDF	Neuroprotection
		In vitro	Mouse N2a cells	OGD				
50.	Moisan et al, (2016)[16]	In vivo	Rat	MCAO	Xenograft	hBM-MSCs (via tail vein)	MRI, FGF, VEGF, SDF1, CXCR4, NeuN, GFAP	Neurogenesis

Table 3 Characteristic of Included Studies (Administration via Intranasal)

No	Author (year)	Study Design	Population			Intervention	Outcome	
			Animal/Cell	Method		Dose	Measure/Marker	Final Effect
1.	Zhou et al, (2023)[8]	In vivo	Mice	tMCAO	Xenograft	Human iPSC-induce MSC Evs ($5x10^{10}$ sEVs)	Ki67, NeuN, DCX, MBP, TrkB, ERK, CREB, CD31, MRI, TTC	Anti inflammatory
2.	Pathipati et al, (2021)[68]	In vivo	Neonatal mice	tMCAO	Allogenic	Mice BMMSC-EVs (1 µg or 5 µg/1 µL) via intracerebroventricular (ICV) injection or intranasally (IN)	Iba1, GLUT1, caspase 3, IL-6, eotaxin, MIP-1α, IL-10, KC, MCP1, MIP-1alfa, Eotoxin	Neuroprotection
		In vitro	Microglial cells from neonatal cortex	Uptake MSC-EVs				
3.	Wei et al, (2015)[69]	In vivo	Rat (neonatal)	pMCAO	Allogenic	Rat BMSCs (1×10^6 cells)	NeuN, collagen IV, Glut-1, GFAP, Behavior test	Neurogenesis

Table 4 Characteristic of Included Studies (Administration via Intracerebral)

No	Author (Year)	Study Design	Population			Intervention	Outcome	
			Animal/ Cell	Method		Dose	Measure/Marker	Final Effect
1.	Yang et al, (2023)[70]	In vivo	Rat	MCAO	Xenograft	HuBMSCs/NC-Exos or BMSCs/miR-133a-3p-Exos via the brain cavity	DAKP2, AKT, Beclin1, mTOR, CCK8, TUNEL, TTC	Neuroprotection
		In vitro	SH-SY5Y	OGD				
2.	Taei et al, (2022)[45]	In vivo	Rat	MCAO	Xenograft	CM hESC-MSC (5 µL in DMEM via Intracerebroventricular)	Bax, Bim, Bcl2, IL-1β, IL-6, IL-10, BDNF, GDNF, NGF, NT-3, CD31 and VEGF	Neuroprotection, neurogenesis and angiogenesis
3.	Lam et al, (2019)[4]	In vivo	Rat	MCAO	Allogenic	Rat AT-MSC ($0.8.10^6$ MSCs applied to ipsilateral parietal cortex)	TTC, GFAP, NeuN and PCNA	Neurogenesis
4.	Fu eta al. (2022)[3]	In vivo	Rat	MCAO	Xenograft	HUMSCs ($0.5x10^6$ transplantation the rat's cerebral cortex)	MRI, TTC, Crystal violet, NeuN, Behavioral Test, GFAP,	Angiogenesis
5.	Yabuno et al, (2023)[71]	In Vivo	Rat	MCAO	Xenograft	hBMMSC - SB623 (0.4×10^6 cells/ 5 µL were stereotactically injected into the right striatum)	Behavioral tests, TTC, NGF, BDNF, VEGF,	Neurogenesis, angiogenesis
6.	Wei et al, (2022)[54]	In Vivo	Rat	MCAO	Allogenic	Zeb2/Axin2-Enriched rat BMSC-Derived Exosomes ($1x10^{11}$/5 µL via lateral ventricles)	Wnt/β-catenin, and endothelin-3/EDNRB, TTC, PCN, BDNF, VEGF, SDF-1, Axin, TGF-β, ZEB2,	Neurogenesis
		In Vitro	Primary Cultured Neuron (PCN)	OGD-Neurit outgrowth				
7.	Kim et al, (2018)[61]	In vivo	Rat	MCAO	Allogenic	Rat BMMSC + Angelica gigas (AG) ($1x10^6$ MSC + 50 mg/kg)	Behavioral test, VEGF, Ang-1, Tie-2, Akt, PI3K, CD31	Angiogenesis
		In vitro	HUVEC	Tube Formation Assay				
8.	Liu et al, (2024)[57]	In vivo	Mice	MCAO	Xenograft	Human iPSC-derived MSCs-Evs, iMSC-sEVs (1×10^{11} particles/500 µL PBS)	GFAP, SO2, DCX, p16, SA-β-gal, and Ki67	Neurogenesis
		In vitro	NSC mice fetus	OGD		1×10^{10} particles/mL concentration		
9.	Bi et al, (2018)[72]	In vivo	MCAO	MCAO+mild hypotermia	Allogenic	Rat BMMSC (1×10^6/ 1 mL PBS were injected into the left lateral cerebral ventricle at the depth of 4.0 mm)	GFAP, VEGF, TTC	Angiogenesis
10.	Faezi et al, (2018)[73]	In vivo	Rat	MCAO	Xenograft	CM-hAMMSC (0,5 µL intraventricular)	Bax, Bcl2, Caspase-3,	Neuroprotection
11.	Aboutaleb et al, (2019)[60]	In vivo	Rat	MCAO	Xenograft	CM-hAM MSC (0.5 µL intracerebral)	HGF, BDNF, VEGF, NGF, ERK1/2, GF-1, angiogenin, IL-8, IL-6, SDF-1 and, HGF	Neurogenesis

(*Continued*)

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Table 4 (Continued).

No	Author (Year)	Study Design	Population			Intervention	Outcome	
			Animal/ Cell	Method		Dose	Measure/Marker	Final Effect
12.	Son et al, (2019)[74]	In vivo	Mice	MCAO	Xenograft	hBMMSC (0. 2 × 10^6/10 μL of neurobasal media via contralateral in the cerebral infarction)	Motor function, GFAP, NeuN, IGF-1, IGFBP-4, Akt, ERK1/2, Bax, Bcl2, Nestin, Vimenti, Sox2, GFAP, Sox10, PDGFRα, SLC1A2, SLC1A3, S100β, PAPP-A, Pax6	Neuroprotective
13.	Kong et al, (2016)[53]	In Vitro	Primary cortical neuron (rat)	OGD	Allogenic	Ratbmmsc (0.5x10^6 MSCs were put in the transwell)	RIP1 and RIP3, Beclin1, caspase-3, AIF	Neuroprotective
14.	Neal et al, (2019)[75]	In Vitro	Primary rat neuronal cells (PRNCs)	OGD/R +Tregs and/or BMSCs	Xenograft	Co-cultured with Tregs and/or BMSCs, at concentrations of either 800, 8000, or 80,000 cells per well	FGF-β, IL-6, Tregs (CD4þ/ CD25þ/FoxP3þ)	Neuroprotection
15.	Wang et al, (2022)[76]	In Vivo	Rat	MCAO	-	MSC transfected BDNF (3 μL hydrogel carrying BDNF-MSCs (1×107 cells/mL) or MSCs (1×107 cells/mL) into the brain)	CK8, CD31, myelin basic protein (MBP), NeuN, IL-1, BDNF, VEGF, IGF-1.	Neurogenesis and angiogenesis
		In Vitro		OGD				
16.	Li et al, (2020)[77]	In vivo		MCAO	Allogenic	Rat BMSCs were transfected by CXCR4 (BMSC-CXCR4) (100 μg/ 5μL Exo, ExoCXCR4, or of PBS was injected into the lateral ventricle of the affected hemisphere)	βtubulin, MTT, SDF-1α, CXCR4, Bax, Bcl3, Cell migration, tube formation	Angiogenesis
		In vitro		The bEnd.3	Cell migration and tube formation			
17.	Fang et al, (2022)[48]	In vitro	HBMECs	OGD/R	Allogenic	BMMSC	SDF-1α/CXCR4, MTT, uPA/ uPAR	Angiogenesis
18.	Lee et al, (2023)[30]	In vivo	Mice	MCAO	Xenograft	Co treatment electroacupuncture (EA) and tenuigenin (TE)+hMSC (0.2 × 10^6/5 μL, were stereotaxically implanted into the left striatum)	DCX, GFAP, NeuN, Sox2, Behavior test	Neurogenesis
19.	Lino et al, (2023)[78]	In vivo	Rat	MCAO	Xenograft	MNC-sEVs and MSC-sEVs (2.29 × 10^9 parts per μg and 3.30 × 10^9 parts per μg of protein via intracerebral)	NeuN. GFAP, Iba-1, CD31, Ki67, DCX, TUNEL	Neuroprotection
20.	Kawauchi et al, (2022)[21]	In vivo	Rat	MCAO	Xenograft	SB623 cells (4.0 × 10^5 cells/5 μL encapsulated SB623 cells, into the right striatum)	DCX, Behavioral tests, STEM101	Neurogenesis
21.	Kim et al, (2018)[9]	In vivo	Mice	MCAO	Allogenic	Mice BMMSC+electroacupuncture (EA) (1X10^5/5μL, were stereotaxically transplanted into the lef striatum)	BDNF, NT4, VEGF, NeuN, Ki67	Neurogenesis
22.	Wei et al, (2022)[25]	In vivo	Rat	pMCAO	Allogenic	FNDC5-overexpressing BMSCs (BMSCs-OE-FNDC5), (1x10^6 cells/ 3 μL DMEM solution) was injected around the infarct)	TTC, LC3, TEM for monitor authophagy, TUNEL, CCK8, Beclin-1, p62, Bcl-2 and Bax	Neuroprotective
		In vitro	Astrocytes and microglia	OGD		EVs (2 × 10^6 cell equivalents for each condition or 13.5 μg EV protein)		
23.	Li et al, (2021)[26]	In vivo	Rat	MCAO	Allogenic	Rat hypoxia BMSCs (2 × 10^5/10 μL of saline were stereotactically injected into the striatum of the ipsilateral hemisphere)	VEGF, IL-1β, TNF-α, caspase-3, LC3, Beclin-1, mTOR, AKT, p62, Bax, Bcl2, BDNF	Neuroprotection
24.	Park et al, (2022)[79]	In vivo	Mice	MCAO	Xenograft	hBM-MSCs+ electromagnetic field (PEMF) (1 × 10^5 cells were injected in saline via the penis, and the cell/ PEMF group was exposed toPEMF (F = 60 hz, 10 mT)	MTT, LDH, MMP-9, TNF-α, IFN-γ, MAP-2, BDNF, ERK, Dcx, Nestin, Behavior test	Neurogenesis

(*Continued*)

Table 4 (Continued).

No	Author (Year)	Study Design	Population			Intervention	Outcome	
			Animal/ Cell	Method		Dose	Measure/Marker	Final Effect
25.	Huang et al, (2018)[80]	In vivo	Rat	MCAO	Xenograft	ADSCs-Exo (100 μg/kg/day was injected via the lateral cerebral ventricle)	TTC, TUNEL, CD63, CD81, PEDF, LC3, p62, caspase-3, caspase-9, TSC101	Neuroprotection
		In vitro	Cell line SH-SY5Y	OGD		10 μg/mL ADSC-Exo		
26.	Yamaguchi et al, (2018)[50]	In vivo	Rat	MCAO	Xenograft	Aged donor hMSC (24 and 64 years old 1 x 10^6 cells/300 mL)	Iba-1, GFAP. RECA-1, Musashi-1 (Msi-1), anti-human cytoplasmic marker STEM, platelet-derived growth factor receptor (PDGFR)-b, TOPRO-3, BDNF	Neuroprotection
27.	Zong et al, (2017)[11]	In vivo	Rat	MCAO	Allogenic	The adenovirus carried VEGF into BMSC (Ad-VEGF-BMSC), and purified adenovirus was transferred into BMSC (Ad-BMSC) (1 × 10^6/ 10 μL of DMEM via intracerebral)	VEGF, BDNF, MAP2	Neuroprotection
		In vitro	The mouse BV2 microglial cells	OGD for 6 hours				
28.	Sun et al, (2020)[29]	In vivo	Rat	pMCAO	Xenograft	HUMSC (VX-765-treated HUMSCs, or VX765 + MHY185-treated HUMSC) (1 × 10^5 / 3 μL to the center of the lesion using a micro-injection needle at a delivery rate of 1 μL/min)	IL-1β, IL-6, IL-10, AMPK, mTOR, Tunnel, MTT. LC3II/ LC3I, Atg5, Beclin-1 and p62	Neuroprotection
29.	Ryu et al, (2019)[81]	In vivo	Rat	MCAO	Allogenic	Rat ATMSC (3D angioarchitecture, transparent, nonsectioned brain)	DCX, Nestin, SOX2, HIF1-α, VEGFα, NG2, PECAM-1, PDGFR-β, Flt-1	Neurogenesis
30.	Park et al, (2017)[46]	In vivo	Rat	MCAO	Xenograft	hUCB-MSCs (5.0×10^5/ 5 μL PBS via intrastriatally)	GFAP, NeuN, TGFB1, COX2, TNFalfa, Tunnel, Laminin, Behavior test	Neurogenesis
31.	Wu et al, (2020)[66]	In vivo	Rat	MCAO	Allogenic	IONlabeled 10^6 MSCs in 10 μL saline were locally injected into the right CC	HGF, IGFBP-3, IGFBP-5, GDF-15, CCL5, CXCL2,IGF	Neurogenesis
		In vitro	Rat MSC	Rat MSC +Choroid Plexus		30–80 ×10^2 rMSCs were seeded on a 50 μg/mL with 0.027 g Choroid Plexus/CP		
32.	Hu et al, (2019)[44]	In vivo	Rat	MCAO	Allogenenic	Rat BMMSC precondition hypoxia 0.5% O2 (1 × 10^5 cells/cm2 for 4, 6, 12, 24, 36, for 48 hours for hypoxic culture)	Tunnel, MTT, Akt, NfkB, caspase 3, caspase8, vimentin, FFOCX2 CXCR4, CXCL12, behavior test	Neurogenesis
33.	Yang et al, (2015)[47]	In vivo	Rat	MCAO	Allogenic	Rat MSC-Exo (400 μg/kg exosomes), rat MSC (5×10^6 /100 μL MSCs), BYHWD (500 μg/kg BYHWD)	VEGF and Ki-67, dicer small interfering RNA (siRNA), miR-126, miR-222, miR-221	Angiogenesis
34.	Jablonska et al, (2016)[64]	In vivo	Rat	MCAO	Xenograft	hUCMSC-NSC 2 μL labeled with CMFDA (2×10^6 via a microinfusion pump)	DCX, MMP 2/9, BDNF, GDNF, NT-3, CNTF, EGF, HGF, and IGF-1	Neurogenesis
35.	Park et al, (2015)[62]	In vivo	Rat	MCAO	Xenograft	hUCB-MSCs (5.0×10^6/ 5 μL PBS intraparenchymally)	GFAP, NeuN, DAPI, laminin, Tunnel	Neurogenesis
		In vitro	Mouse N2a	OGD				
36.	Li et al, (2019)[19]	In vivo	Rat	MCAO	Allogenic	Rat BMMSC + Transient receptor potential canonical (TRPC) (1 × 10^6/10 μL PBS)	Btubulin, Tunnel, TRPC,	Neurogenesis
37.	Li et al, (2019)[82]	In vivo	Rat	MCAO		MSC (MSC-shRNA-NC or MSC-shRNA-SNHG12) 2x10^6/200 μL via stereotactically)	TTC, Tunnel, caspase 3, LC3BI, LC3BII, p62, Akt, mTOR, PI3K	Neuroprotective
		In vitro	Rat BMECs	BMEC				
38.	Nouri et al, (2015)[18]	In vitro	Neuron-hWJMSC	H2O2+ Deferoxamine		h WJ-MSC	BDNF, VEGF, Bax, Bcl2, Akt1	Neurogenesis

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Discussion

This scoping review examined the therapeutic effects of employing MSCs, secretome, or EVs in stroke models. The effects on angiogenesis, neurogenesis, and neuroprotection were verified using a variety of indicators. Our scoping review contributes significant discoveries to the present literature. We discovered a small number of papers on neuroprotective benefits via the autophagy pathway.[12,29,42,45,67,70,73,74,86,87] Anti-inflammatory is one of the neuroprotective benefits described by various researchers because cytokines, as immunomodulators, help to suppress inflammation.[33,39,52,53,79,88,89] Anti apoptosis is more typically associated with neurogenesis effect although it is also related to the anti-inflammation and autophagy process.[38,40,44,74,90–92]

Interestingly, we discovered a variety of articles reporting diverse methods for increasing the potency of MSCs, including 3D synthesis, hypoxic settings, and gene transfection.[31,32,93] This is done to boost the therapeutic efficacy of BDNF, CircAkap7, AxCALacZ-F/RGD, and CCL2 transfection therapy, which is intended to improve migration, neurogenesis, autophagy, and oxidative stress.[39,48,77] However, the neuroprotective impact is primarily explained by anti-inflammatory and apoptotic pathways, with little studies on the effects of autophagy and antioxidants. Post-reperfusion in ischemic stroke can cause neuronal cell death owing to oxidative stress, hence further research is needed on the effects of boosting endogenous antioxidants to enhance the neuroprotective impact.[42,47,82,94]

To stimulate the release of bioactive MSCs, the researchers treated them with hypoxia, stroke patients serum and herbal medicine, which increased the therapeutic efficacy via migration and neurogenesis processes.[30,50,60,62,66,95,96] Ferulic acid in the herbal content is supposed to help create a microenvironment that promotes cell survival, migration, and differentiation, as well as tissue connections, making it effective for mending brain tissue and recovering from an ischemic stroke.[49,51,61,64,97–99] Herbal or antioxidant administration can be combined, although some practitioners use herbal extracts to boost the secretome's bioactivity, such as promoting progenitor neuron migration to the infarct location.[66,71,72,100–102] Bioactive neurotrophic factors derived by MSCs, like as BDNF, have also been shown to trigger neurogenesis from endogenous progenitor neurons.[63,77,103,104] However, Zhang et al (2023) found that MSC therapy induces angiogenesis and oligodendrocytes, which are involved in the axon myelination process, allowing electrical signals to surface swiftly.[34]

Mesenchymal stem cell/MSC also have a function in post-ischemic angiogenesis in the infarction location, allowing neuroprotection to occur.[27,35,36] Inducing angiogenesis with herb administration and exercise to improve post-ischemic stroke healing, which is influenced by enhanced miRNA production or functioning synergistically in endothelial progenitor migration.[41,54,68,78,105] Bioactive MSCs also boost the activity of VEGF, MMP-2, and MMP-9, which all contribute to increased blood vessel density.[57,58] Although animal research with stroke comorbidities such as hypertension, atau aged revealed that MSC therapy did not produce positive benefits.[43,46]

Extracellular vesicles produced by MSCs represent a novel biomarker and effective target therapy for ischemic stroke. These vesicles are microscopic particles attached to a lipid bilayer that allow intercellular communication and transport a variety of bioactive substances such as proteins, lipids, and RNA, to increase delivery to target cells, modification is given by adding cholesterol as drug delivery.[59] EVs promote neurogenesis by upregulating microRNAs leading to brain tissue regeneration.[15,56] Reducing inflammation by modifying the immune response, EVs may help reduce inflammation in the brain following a stroke.[69,106] EVs can also pass the blood-brain barrier, which allows them to deliver therapeutic drugs directly to damaged brain tissue, enhancing their effectiveness.[81,107] The inflammatory process also initiates the processes of neurogenesis and angiogenesis, thus helping restoration in the penumbra area.[15,37,55,56,108,109]

This study has significant limitations, including the lack of studies comparing MSC therapy to CM or EVs, as well as control and MCAO treatment. According to prior systematic evaluations, treatment groups using MSC, CM, or EVs had considerably better functional and biomechanical outcomes than control groups. In vitro studies revealed that the treatment group exhibited therapeutic effects in angiogenesis, neurogenesis, and neuroprotection. In vitro research has the benefit of being more controlled than in vivo experiments; yet, the autophagy effect continues to occur in vivo, resulting in discrepancies in outcomes. The fact that they all report varying concentrations and non-uniform delivery routes is concerning. Administration via the cerebral route has the benefit of reaching directly to the target organ, is performed by a specialist, and requires fewer MSCs and secretome.[110] The intra-arterial route is also more intrusive than

the intravenously approach, although the intra-nasal route may be a possibility if the patient is also comfortable.[4,8,28,111–113]

This study used the idea of xenografts rather than allogeneic or autologous, hence the variances in outcomes are also distinct.[3,65,114] In addition, the use of animal models such as rats and mice, which have very different blood circulation from humans, creates obstacles in becoming a reference for translational research. The limitations of animal models are also a barrier to translational research. Non-human primates can be used as animal models. Administration via intranasal, which is non-invasive but close to the target organ, is also an option for more comfortable delivery.

Finally, more research is needed to investigate the use of animal models such as non-human primates, concentration and dosage of MSC, secretome, and EVs to accomplish effective therapy. Information on the role of MSC-secreted miRNAs as therapeutic targets. Obtaining thorough knowledge regarding these aspects can improve therapy effectiveness, hence contributing to advancement in stroke therapy.

Limitations

This study did not conduct a critical appraisal of the included studies and differences in the way data were reported in the articles. A systematic and comprehensive search was conducted, but only articles written in English were included, which limits the applicability of the review results to the English-speaking world. This study also provides insight into the obstacles and challenges of stem cell therapy research for stroke, especially to be a reference for translational research towards clinical trials. Limitations between articles are variability in sample size, study design, and outcome measures, which may affect the generalizability and comparability of the results. To overcome this, more rigorous future studies with standardized outcome measures are needed so that clinical practice findings can be a strong basis for the success of clinical trials.

Conclusion

In conclusion, MSC and secretome therapy for stroke has significant potential in both in vitro and in vivo models. Current research shows the potential of MSC and its secretome to increase neurogenesis and neuroprotection but the challenge is the gap in understanding to conduct translational research in optimizing stroke treatment. Exploration of various sources of MSC, refining isolation techniques, transfection treatments of various proteins, combinations with herbal medicines are efforts to improve preclinical models. Understanding of signaling pathways, mechanistics, safety, and effectiveness in the preclinical stage has been obtained so that further research is needed for clinical trials.

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