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REVIEW

Neuroprotective Effects of Conditioned Medium of Mesenchymal Stem Cells (MSC-CM) as a Therapy for Ischemic Stroke Recovery: A Systematic Review

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Abstract

It has been reported that the therapeutic potential of stem cells is mainly mediated by their paracrine factors. In order to identify the effects of conditioned medium of mesenchymal stem cells (MSC-CM) against stroke, a systematic review was conducted. We searched PubMed, Scopus, and ISI Web of Science databases for all available articles relevant to the effects of MSC-CM against the middle cerebral artery occlusion (MCAO) model of ischemic stroke until August 2022. The quality of the included studies was evaluated using The STAIR scale. During the systematic search, a total of 356 published articles were found. A total of 15 datasets were included following screening for eligibility. The type of cerebral ischemia was the MCAO model and CM was obtained from MSCs. The results showed that the therapeutic time window can be considered a crucial factor when researchers use MSC-CM for stroke therapy. In addition, MSC-CM therapy contributes to functional recovery and reduces infarct volume after stroke by targeting different cellular signaling pathways. Our findings showed that MSC-CM therapy has the ability to improve functional recovery and attenuate brain infarct volume after ischemic stroke in preclinical studies. We hope our study accelerates needed progress towards clinical trials.

Keywords Ischemic stroke · Conditioned medium of mesenchymal stem cells · Functional recovery · Molecular mechanisms · Angiogenesis · Outgrowth · Neurogenesis

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Introduction

Stroke is known as a seriously debilitating disorder and one of the main reasons of death for people older than 60 years of age [1–4]. It is characterized by symptoms of brain ischemia and hemorrhagic injury and has been considered a major challenge due to economic burden and mental pressure to society and the family [5–8]. The discovery of novel therapeutic agents with high efficiency and safety for treatment of stroke is a fundamental issue that requires significant attention due to the lack of translation of a wide range of them into human use [2, 9–12]. Although endovascular intracranial thrombectomy (EVT) and thrombolysis with recombinant tissue plasminogen activator (rt-PA) are the two major treatments for the acute stage of ischemic stroke, their clinical applications have been limited owing to their narrow therapeutic window (rt-PA, < 4.5 h; EVT < 6 h) [13, 14]. Additionally, rt-PA might have some side effects such as increasing inflammatory response in brain capillaries and consequently neuronal cell death [15–17]. Therefore, there is an urgent need to explore new therapeutic agents or materials with highest efficiency and lowest adverse effects. New therapeutic agents and techniques should have ability to amplify endogenous processes for tissue regeneration such as angiogenesis, neurogenesis, and axonal outgrowth [18]. Stem cell based therapy has been known as a promising strategy to attenuate neurological disorders such as cerebral ischemia/reperfusion injury and improve life quality of patients [19–24]. Mesenchymal stem cells (MSCs) have attracted tremendous interest in treatment of ischemic diseases such as stroke owing to their unique properties such as ability for modulation of the inflammatory responses, low immunogenicity, easy attainability, and differentiation plasticity [25–31]. Aside from benefits of MSCs, their extensive use for treatment of stroke has been challenged by concerns regarding immunogenicity, route of administration, dosage, migration to other body organs, and their unwanted differentiation [32, 33]. Many previous studies have reported that neuroprotective effects of MSCs against ischemic stroke are relevant to trophic factor secretion by them [34, 35]. In fact, MSCs have demonstrated ability to secrete neurotrophic and immune modulatory factors which play an important role in rehabilitation of damaged tissue [36]. Although Researchers firstly hypothesized that the action mechanism of MSCs is due to cell replacement, currently it was found that their paracrine actions or "bystander" effects of MSCs is responsible for regeneration [37]. Conditioned medium of MSCs (MSC-CM) has a wide range of proangiogenic and antiapoptotic factors like angiogenin, exosomes, interleukins, and hepatocyte growth factor [38]. In this systematic review, we highlighted recent insights into the role of conditioned medium of MSCs and its effects changing molecular mechanisms in nerve regeneration and healing processes after stroke. In addition, potential applications of MSC-CM for treatment of ischemic stroke are reviewed.

Methods and Material

This systematic review was designed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [30].

Literature Search

The articles used for this review were selected from Pubmed and Medline, for in vivo models of stroke until August 2022. We conducted the PUBMED search using the following search terms: ("conditioned medium" or "conditioned media") and ("stroke" or "cerebral ischemia" or "brain ischemia" or "middle cerebral artery occlusion" or "MCAO" or "cerebral infarct"). Titles of the search result were screened by two investigators to find the eligible articles. After the first screening process, the same investigators applied inclusion and exclusion criteria to each article. The final list of references was checked by a third investigator who was an expert in the field.

Inclusion and Exclusion Criteria

In the second screening process, the articles should have had these criteria to be included in this review: in-vivo studies; induced ischemic stroke, brain ischemia, or brain hypoxia; treatment with MSC-CM; evaluation of both lesion size changes and neurological/behavioral functions; studies that used a control group; original publications. The applied exclusion criteria were as followed: human studies; in-vitro and in-situ studies; no induced ischemic stroke; cerebral hemorrhagic models; not measuring lesion size changes; not testing the neurological/behavioral functions; studies presented in form of a review, conference abstracts, letters, and abstracts; abstract not in English; and studies investigating the effect of exosomes or MSCs directly.

Data Extraction

From each selected article, we recorded author, journal, publication year, country, ischemic model, type of animal (species, strain, sex, age, and weight), treatments use (density of the CM, dosage), and outcomes. The outcomes were categorized as lesion size, neurological/ behavioral functions, brain repair markers, and angiogenesis markers. Significance was considered when the P-value was less than 0.05.

Quality Assessment

The quality score that estimates methods, defined 10 criteria based on Stroke Therapy Academic Industry Roundtable (STAIR) guidelines and is being used for each preclinical study to review the animal data from the experimental studies checklist [31, 32]. The criteria are as followed; (1) publication in a peer-reviewed journal, (2) statements describing control of temperature, (3) random assignment of animals to treatment group, (4) allocation concealment, (5) blinded outcome assessment, (6) avoidance of anesthetics with known marked intrinsic neuroprotective properties, (7) use of animals with relevant comorbidities, (8) inclusion of a sample-size calculation, (9) statement of compliance with animal welfare regulations, and (10) inclusion of a statement declaring presence or absence of any conflicts of interest. One point was given for each criterion reported. The potential score ranges from 0 to 10, with higher scores indicating greater methodological rigor (Table1).

Results

356 non-duplicate publications were identified. 312 publications were excluded after title and abstract screening, and 29 publications were further excluded after full-text screening. The remaining 15 studies were included in this systematic review (Fig. 1).

Subjects in Included Studies

All 15 included studies were animal studies, involving mice in one and rats in 12 (Table 2). In them, a transient middle cerebral occlusion (tMCAO) model for 15, 20, 60, 90, and 120 min was used in 1, 1, 8, 2, and 4 studies, respectively. The sources of exosomes were bone marrow stromal cells (BMSCs), adipose-derived mesenchymal stem cells (ADMSCs), human Amniotic mesenchymal stem cells (hAMSCs), human embryonic MSCs, human limbus stromal cells, IL-1α-primed MSC, human umbilical cord MSCs (hUCMSCs) in 4, 3, 3, 2, 1, 1 and 1 studies, respectively. Timing of conditioned medium administration varied from 1 h before ischemia or immediately to 24 or 48 h after reperfusion. Behavioral assessments including the modified neurological severity score (mNSS), the foot-fault test, grasping power test, neurological scores, beam balance test (BBT),corner test, open field test,. rotarod test, Garcia scale test, cylinder test, point neurological score, burrowing behavior, nest building, were performed, and the maximum time of evaluation of motor function was 1–28 days.

Quality Score

10 out of 15 studies obtained a score of 5 or higher. All studies have been published in peer-reviewed journals. 10 studies reported describing control of temperature, 11 of 16 studies reported randomized allocation to treatment group, only 7 studies reported blinded assessment of outcome and mentioned avoidance of anesthetics with known marked intrinsic neuroprotective properties, none of them used animals with relevant comorbidities or reported a sample size calculation and randomized allocation to treatment group, 11 studies stated compliance with animal welfare regulations, and 12 studies stated possible conflicts of interest (Table 1).

Therapeutic Effect of Conditioned Medium Derived from MSCs Stroke Recovery

In a study by Cho and colleagues, using Sprague–Dawley rats with tMCAO for 60 min, human adipose stem cell-conditioned medium (hADSC-CM) were injected 7 days after stroke via the stereotaxic administration, and the rotarod test was performed 3 days before MCAO and 7 days and 15 days after MCAO to measure functional performance. The treatment group showed significantly better performance on the rotarod test compared with the control group (15 days after MCAO $P < 0.05$). At 15 days after the MCAO surgery, infarction volumes were calculated by TTC staining. The treatment group showed reduction of the infarction volume compared with the control group (control, mean ± SEM = 255.03% ± 16.83%; treatment, 202.43% ± 33.03%), although it was not statistically significant. Using tube-formation assay, tube length increased dose-dependently and significantly with the addition of ahADSC-CM ($p < 0.005$); the treatment group also had a—not statically significant- reduction in the infarct area compared to the contralateral hemisphere; they also showed an increase in CD31-positive microvessels, compared with the control group (control, mean ± SEM = 100.20 ± 7.51; treatment, 130.47 ± 21.54; $P < 0.05$), which confirms the angiogenesis ability of ahADSC-CM. Neuroprotective effects of ahADSC-CM could also make neural cells resistant to apoptosis from hypoxia in the penumbra region. The number of TUNEL-positive cells was significantly reduced by the continuous infusion of ahADSC-CM. These data show that ahADSC-CM improves motor recovery after ischemic stroke, along with an elevation in angiogenesis and a reduction in lesion size.

Liang and colleagues [40] examined the neurotrophic and neuroprotective effects of conditioned medium from limbus stroma-derived mesenchymal stromal cells (L-MSCs) in focal cerebral ischemia in male Sprague–Dawley rats. Conditioned medium was produced by culturing human L-MSCs under normoxic (CM-N) and hypoxic (CN-H) conditions.

Table 1 Quality check of pre-clinical studies using STAIR guideline

Author, year (reference)	Publication in a peer-review journal	Control of temperature	Random assignment	Allocation concealment	Blinded outcome assessment	Avoidance of anesthetics with known marked intrinsic neuroprotective properties	Use of animals with relevant comorbidities	Inclusion of a sample-size calculation	Compliance with animal welfare regulations	Presence or absence of any conflicts of interest
[39]	1	1	1	0	0	1	0	0	1	0
[40]	1	1	0	0	0	1	0	0	1	1
[41]	1	1	1	0	0	0	0	0	1	1
[42]	1	0	0	0	1	0	0	0	1	1
[43]	1	1	1	0	0	1	0	0	1	1
[44]	1	1	1	0	1	1	0	0	1	0
[45]	1	1	1	0	0	0	0	0	1	1
[46]	1	0	0	0	0	0	0	0	1	0
[47]	1	0	0	0	1	0	0	0	0	1
[48]	1	1	1	0	1	1	0	0	1	1
[36]	1	1	1	0	0	1	0	0	1	1
[49]	1	0	1	0	1	0	0	0	0	1
[50]	1	1	1	0	1	1	0	0	0	1
[51]	1	0	1	0	1	0	0	0	0	1
[52]	1	1	1	0	0	0	0	0	1	1

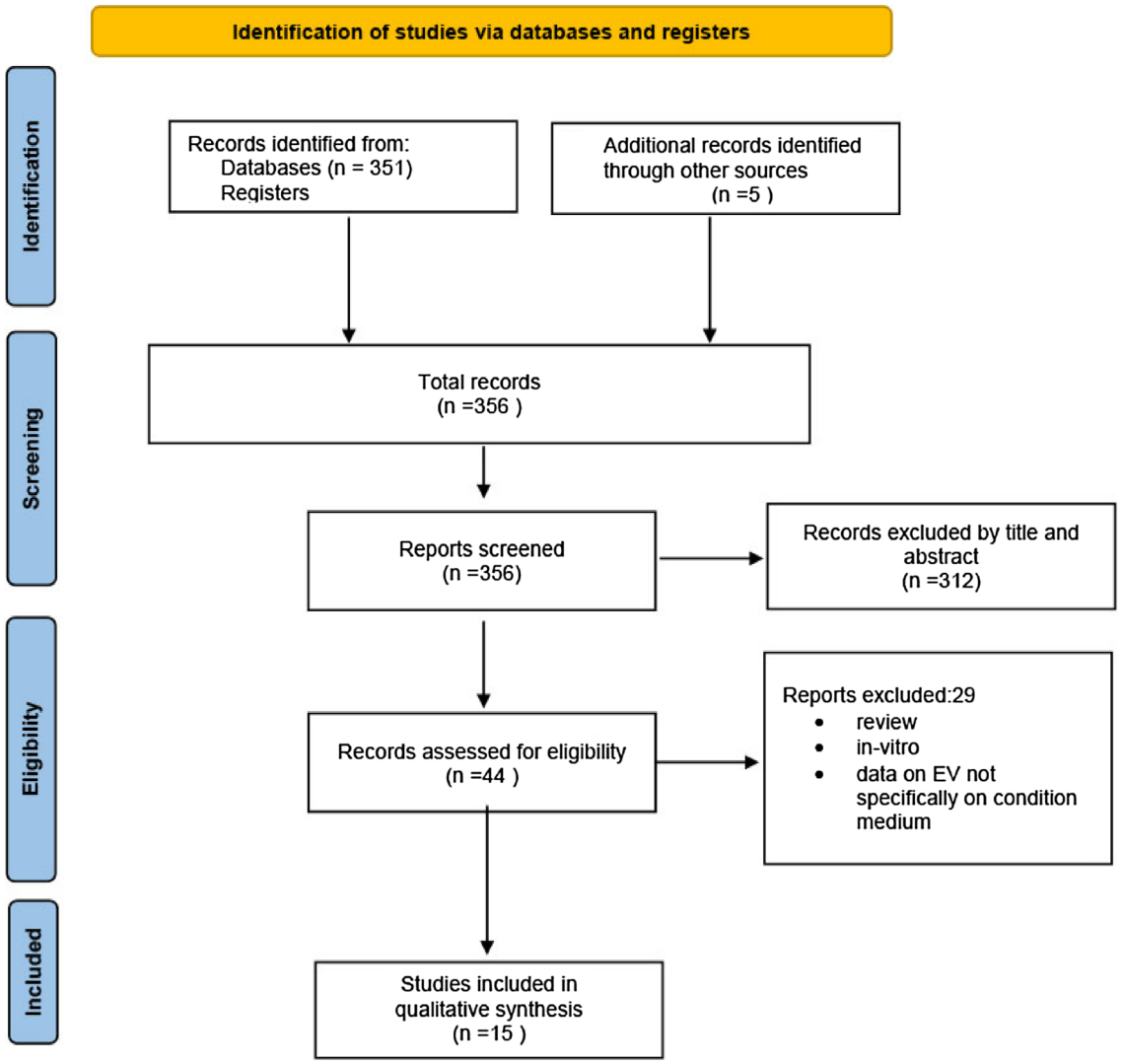

Fig. 1 Flow chart of the study selection. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram shows the number of records identified, included, and excluded through the different phases of a systematic review

Using a rat model of tMCAO for 90 min, treatment with CM-H reduced infarct volume and motor function on open field test more that CM-N. Both CM-H and CM-N were rich in factors like insulin-like growth factor-2 (IGFBP2), VEGF, VEGFR3, IGF2, HGF and granulocyte macrophage colony-stimulating factor (GM-CSF), but hypoxia significantly induced an increase in VEGF and BDNF expression levels, whereas other factors (IGF2 and HGF expression) declined in this condition, which might be the reason for better improvement in CM-H.

Egashira et al.[44] studied the effects of human and murine adipose-derived stem cells' conditioned medium (hASC-CM and mASC-CM respectively) in mice after 2 h of MCAO followed by 22 h reperfusion. In the first experiment, they pretreated the mice with a single i.c.v. injection of 2 µl of tenfold, 30-fold, or 100-fold concentrated mASC-CM, 1 h before the MCAO. Both 30 and 100 fold reduced infarct volume, infarct area, brain swelling, and neurological score. They also assessed the therapeutic time window, and found that mASC-CM can significantly reduce infarct volume and area if it's injected with a 5 min delay after MCAO; but when they injected the CM 2 h after MCAO, its effect was not significant. In another experiment they pretreated the mice with hASC-CM, in which only the 100 fold concentration caused a reduction in infarct volume and infarct area, but none of the injections made a difference in brain swelling.

Seo and colleagues [49] studied the neuroprotective effect of hASC-CM, in a rat ischemic stroke model. They treated rats subjected to tMCAO for 2 h with hASC-CM at 1 h after reperfusion. At 24 h post-MCAO, the CM group showed significantly better sensorimotor neurological test scores

Table 2 Summary of characteristics included studies

Authors, year	Animals (species, weight)	Model of stroke, duration of ischemia (Min)	Therapeutic intervention by mesenchymal conditioned medium, type of administration, timing, dosage	Source of mesenchymal conditioned medium	Outcome	Maximum Follow up_day
Egashira Y et al., 2012	Male ddY mice (22 to 30 g)	tMCAO, 120	i.c.v. injection, 1 h before ischemia, 5 min or 2 h after ischemia, 4×10^5 MSCs released	Human and murine adipose-derived stem cells	1. Neurological scores 2. Cerebral infract volume	1Day
Tsai MJ et al., 2014	Male Long Evan (LE)rats (250–350 g)	tMCAO, 60	IV injection, Immeadiately after reperfusion, 1.077 g/ml	BMSCs	1.grasping power test 2.Cerebral infract volume	7Day
Faezi M et al., 2018	Male Wistar rats (250–300 g)	tMCAO,60	Intraventricular injection injection, 30 min after reperfusion, 1×10^6 particles	hAMSCs	1. Neurological scores 2.Beam balance test (BBT) 3. Corner test 4. Cerebral infract volume	1Day
Xiang J et al., 2017	Male Wistar rats (225–250 g)	tMCAO, 120	IV injection, 24 h after reperfusion, 10 ml/kg/rat	BMSCs	1. mNSS 2.Foot-fault test	14Day
Liang CM et al., 2014	Male spragus-Dawley rats (450-600 g)	tMCAO, 60	intracerebral injection, Ninety minutes before reperfusion, approximately 2×10^5	Human limbus stromal cells	1. Open field test 2. Cerebral infract volume	3Day
Zhao Q et al., 2015	Male Wistar rats (225–250 g)	tMCAO, 120	Intranasal administration,24 h after ischemia, 1 ml/kg/d	hUCMSCs	1. mNSS 2..Foot-fault test 2. Cerebral infract volume	14Day
Aboutaleb N et al., 2019	Male Wistar rats (250–300 g)	tMCAO, 60	Intraventricular injection, 30 min after reperfusion, 1×10^6 particles	hAMSCs	1. mNSS 2..Rotarod test 3. Cerebral infract volume	1Day
Cho YJ et al., 2012	Male spragus-Dawley rats (8-week-old)	tMCAO, 60	1, 24, and 48 h following induction of MCAO,4.2×10^7	Human Adipose stromal cells	1..Rotarod test 2. Cerebral infract volume	18Day
Seo HG et al., 2017	Male spragus-Dawley rats (220–300 g)	tMCAO, 120	intracerebroventricularly injection,1 h after reperfusion, NS	Human Adipose stromal cells	1.Garcia scale test 2.foot fault test 3. Rotarod test 4. Cerebral infract volume	1Day
Jiang RH et al., 2019	Male spragus-Dawley rats (240–270 g)	tMCAO, 60	IV injection, 12 h after surgery, NS	BMSCs	1. mNSS 2. Beam balance test 3. Open field test 4. Cerebral infract volume	28Day
Taei AA et al., 2021	Male Wistar rats (260–290 g)	tMCAO, 90	intracerebroventricularly injection. 1, 24, and 48 h following induction of MCAO 1×10^6 MSCs released	Human embryonic MSCs	1. cylinder test 2. Cerebral infract volume	7Day

Table 2 (continued)

Authors, year	Animals (species, weight)	Model of stroke, duration of ischemia (Min)	Therapeutic intervention by mesenchymal conditioned medium, type of administration, timing, dosage	Source of mesenchymal conditioned medium	Outcome	Maximum Follow up_day
Nazarinia d et al., 2019	Male Wistar rats (270–300 g)	tMCAO, 60	IV injection, Immeadiately after reperfusion, 1×10^6 particles	hAMSCs	1. Neurological scores 2. Cerebral infract volume	1Day
Cunningham CJ, 2020	Male C57BL/6 mice	tMCAO, 20 (study1),15 (study2)	subcutaneous injection, 24 h after reperfusion or at the time of reperfusion, 3.5×10^5 particles	IL-1α-primed MSC	1. point neurological score 2. Rotarod test 3. Open field test 4. Burrowing behaviour 5. Nest building	14Day
Tsai MJ et al., 2021	Male LE rats (250–350 g)	tMCAO, 60	IV injection, 2 h, 1, and 2 days for three consecutive days after MCAO, 1.077 g/ml	BMSCs	1. neurological deficit score test 2. grasping power test 3. Cerebral infract volume	7Day
Taei AA et al., 2021	Male Wistar rats (260–290 g)	tMCAO, 90	intracerebroventricularly injection, 1, 24, and 48 h following induction of MCAO 1×10^6 MSCs released	Human embryonic MSCs	1. mNSS 2. Bederson's test 3. Cerebral infract volume	7Day

SD Sprague Dawley, *tMCAO* transient middle cerebral artery occlusion, *IV* intravenous, *MSCs* mesenchymal stromal cells, *BMCSs* bone marrow mesenchymal stromal cells, *ADMSC* adipose derived mesenchymal stem cell, *mNSS* modified neurological severity score, *NS* Not Specified, *hAMSCs* human Amniotic mesenchymal stem cells, *hUCMSCs* human umbilical cord mesenchymal stem cells

than the control group. The infarct volume was significantly lower in the CM group than in the control group. The number of TUNEL-positive apoptotic cells was reduced, whereas HSP70 expression was enhanced in the peri-infarct area in the CM group. Moreover, hASC-CM reduced IκB phosphorylation and influenced bcl-2 and bax protein expression. Furthermore, they reported that IL-6, VEGF, HGF, and BDNF were detected in hASC-CM. The neuroprotective effects of the hASC-CM appear to be mediate by an anti-inflammatory mechanism and cell apoptosis inhibition.

TSAI et al. [47] conducted another study using the adult male Long Evan (LE) rats and showed that application of CM obtained of bone marrow mesenchymal stem cells derived from normal and cerebral ischemia rats significantly improved motor function on grasping power test. Other data shows that NormBM-MSC Cm and IschBM-MSC Cm substantially increased neuronal progenitor cell surrounding lateral ventricle in stroke-affected hemisphere. NormBM-MSC Cm and IschBM-MSC Cm also significantly attenuated microglia/macrophage infiltration in the ischemic brain. Enhancement of neurogenesis and attenuating microglia/macrophage infiltration may contribute to improvement of functional outcome.

Xiang and colleagues [42] studied the effects of bone marrow stromal cells conditioned medium (BMSCs-CM) treatment after stroke in type 2 diabetic (T2DM) rats. Using a rat model of MCAO for 2 h, BMSCs-CM (10 ml/kg) were injected 24 h after MCAO via intravenous injection, and neurophysiological analyses such as the foot-fault test and modified neurological severity score (mNSS) were conducted before MCAO, at 7 and 14 days after MCAO. Treatment with BMSCs-CM after stroke in T2DM rats significantly improved functional outcomes, decreased BBB leakage, increases vascular remodelling. It was also suggested that enhanced expression of angiopoietin (Ang) 1 and tyrosine kinase (Tie) 2 in ischemic brain after BMSCs-CM treatment of stroke may contribute to the improved functional recovery after stroke in T2DM rats.

Furthermore, Zhao et al. [39] reported that treatment with human umbilical cord mesenchymal stem cells' conditioned medium (hUCMSCs-CM) 24 h after reperfusion (1 ml/kg/d, intranasal routine) on foot-fault test and mNSS promotes functional outcome at 7 and 14 days after ischemia. Angiogenesis and angiogenic factor expression were measured by immunohistochemistry, and Western blot, respectively. The hUCMSCs-CM treatment of stroke by intranasal routine starting 24 h after MCAo in rats significantly enhances BBB functional integrity and promotes functional outcome but does not decrease lesion volume compared to rats in control group and saline control group. Treatment of ischemic rats with hUCMSCs-CM also significantly decreases the levels of Ang2 and increases the levels of both Ang1 and Tie2 in the ischemic brain, may be contributed to vascular remodeling in the ischemic brain which plays an important role in functional outcome after stroke.

In a study by our group [41], in order to induce focal cerebral ischemia in rats, MCAO was occluded for 1 h and the human amniotic mesenchymal stem cells-conditioned medium (hAMSC-CM) at the dose of 0.5 µl was administered 30 min after reperfusion by stereotactic intracerebral infusion. On immunohistochemistry, expression of BDNF, VEGF and NGF significantly decreased in MCAO rats and reversed by hAMSC-CM, indicating that rat hAMSC-CM facilitated neurogenesis and angiogenesis in the sub-acute phase of stroke. Likewise, AMSC-CM markedly reduced neuronal loss and DNA fragmentation at 24 h after reperfusion. Two behavioral tests were carried out to evaluate the functional deficits over the first 3 days following MCAO. The mNSS results showed that administration of hAMSC-CM resulted in lower scores compared with MCAO. Additionally, hAMSC-CM treated rats revealed better results in rotarod test. On the other hand, post treatment with hAMSC-CM significantly reduced infarct volume and brain edema but increased BBB integrity. Treatment with AMSC-CM at the onset of reperfusion markedly increased the phosphorylation of ERK1/2 in the cortex area of infarcted rats and also significantly promoted neuronal survival through overexpression of Bcl-2 and subsequently inhibition of apoptosis. In this study, it was suggested that AMSC-CM might exert neuroprotective effects through activation of neurogenesis in an ERK1/2- BDNF pathway dependent mechanism.

In another study by our group [45], we studied anti-apoptotic effects of hAMSC-CM for focal cerebral ischemia. The rats subjected to tMCAO for 60 min were treated with hAMSC-CM at 30 min after reperfusion. Treatment with hAMSC-CM significantly improved neurological deficits and motor coordination on beam balance test (BBT) at 24 h after reperfusion. Immunohistochemistry showed that the expression of Bax and caspase-3 was markedly increased and the expression of Bcl-2 was significantly decreased following occlusion of MCA and were reversed by hAMSC-CM. Thus, hAMSC-CM exerts neuroprotection against focal cerebral ischemia by targeting apoptosis.

Jiang and colleagues [50] examined the hypoxic CM derived from bone marrow mesenchymal stromal cells protects against ischemic stroke in rats. Using a rat model of tMCAO for 1 h, hypoxic and normoxic CM were injected through the tail vein 12 h after surgery on day 0 and then every 2 days thereafter for 28 days, and neurophysiological analyses such as mNSS and balance beam test were performed before MCAO and on days 1, 7, 14, 21, and 28 after stroke. The CM groups, particularly the hypoxic CM group, showed significant improvements in the mNSS and balance beam test compared with the DMEM group beginning on day 7 after MCAO. At 48 h after MCAO, infarct area in the normoxic groups was significantly decreased compared

with that in the DMEM group, and hypoxic preconditioning enhanced this effect. Both immunofluorescence and TUNEL were used to examine cell apoptosis in the peri-infarct region of rats on day 3. The maturation of cleaved-caspase-3 (apoptosis related marker) was significantly lower in the hypoxic CM group than the DMEM group. Treatment with CM especially the hypoxic CM significantly increased expression of vWF (vascular endothelial cell marker) and VEGF on day 28 after stroke, suggesting enhanced angiogenesis in the peri-infarct tissue. The results suggested that hypoxic CM administration significantly increased the protein levels of PI3K and p-Akt in the ipsilateral hemisphere of rats compared with the levels observed in the DMEM group, and the effect was obviously greater than that observed after normoxic CM administration. These results indicated that the beneficial effects of hypoxic CM on ischemic stroke partly resulted from the activation of the PI3K/Akt signaling pathway. In this study, it was suggested that administering CM after ischemic stroke reduces infarct area and promotes neurological recovery via the attenuation of apoptosis and acceleration of neovascularization.

In another study [52], our group evaluated the effects hAMSC-CM in focal cerebral ischemia/reperfusion in Wistar rats. Treatment with hAMSC-CM immediately after cerebral reperfusion (i.v) significantly improved motor function at 24 h after tMCAO. Compared with sham, significant infarct volume, apoptotic cell death, and neuronal loss were found in MCAO rats that reversed by hAMSC-CM ($P < 0.05$). Likewise, MCAO rats exhibited an increased mRNA level of lightchain 3 (LC3) and the LC3II/LC3I ratio as well as decreased expression level of p-mTOR that reversed by hAMSC-CM ($P < 0.05$). There were no significant differences in the expression of total mTOR among the experimental groups. These results demonstrated that hAMSC-CM gives rise to neuroprotection following ischemic stroke by restoring mTOR activity and inhibiting autophagy.

Tsai et al. [51] studied the effect of monotherapy with CM derived from BMSCs (BM-MSCcm) and combination therapy of BMSC-generated conditioned medium and minocycline in Wistar rats subjected to transient MCAO. The in vitro experiment demonstrated that BM-MSCcm and combined treatment both significantly increased neuronal connection and oligodendroglial numbers and minocycline and combined therapy were both effective in reducing H_2O_2- or LPS-induced free radical levels in cortical neuron–glial cultures. In vivo results showed that combination therapy with BM-MSCcm and minocycline decreased infarction volume and functional behaviors in rats. Test was performed before and at 1 and 7 days post-injury by the neurological deficit (ND) score test and the grasping power test. The combined therapy significantly improved grasping power, which was not altered by monotherapy. NDS was significantly decreased in the MSCcm-treated group at 1 day post-MCAo but did not differ among the control, BM-MSCcm, and minocycline-treated groups at the end of 7 days post-MCAO. They also found that combined treatment had a tendency to increase neuroprogenitors in the corpus callosum and significantly attenuated microglia/macrophage infiltration in the ipsilateral ischemic cortex of MCAo rats. Furthermore, the combined therapy increased the expression of NeuN in the rat brain peri-infarct zone and hippocampus. This study concluded that BM-MSCcm in combination with minocycline promoted neuroprotection, improved neurological functional outcome and also reduced cerebral infarction through antioxidant and anti-inflammation activities.

In the study by Cunningham et al. [48] stroke was induced in male C57BL/6 mice using the intraluminal filament model of MCAO. CM from IL-1α-primed MSCs or vehicle was administered at the time of reperfusion or at 24 h post-stroke by subcutaneous injection. They showed that IL-1α-primed MSC-CM treatment at the time of stroke led to a ~30% reduction in lesion volume at 48 h after MCAO and improved neurological score at day 2 post-MCAO.

Asgari Taei et al. [18] investigated the effect of CM derived from human embryonic MSCs on experimental ischemic stroke. CM was infused either one time (1 h post-MCAO) or three times (1, 24, and 48 h post-MCAO) through guide cannula into the left lateral ventricle. Neurological functions were evaluated using Bederson's test and modified neurological severity score on days 1, 3, and 7 following MCAO. Infarction volumes and cerebral edema were measured on days 3 and 7. The results indicated that three times injections of CM could significantly reduce mortality rate, infarct volumes, cerebral edema, and improve neurological deficits in MCAO rats. Moreover, three injections of CM could restore decreased levels of synaptic markers in MCAO rats up to its normal levels observed in the sham group. This data suggested that using the CM obtained from embryonic stem cells–MSCs could be a potent therapeutic approach to attenuate cerebral ischemia.

Discussion

In the present study, a systematic review was conducted to clarify the therapeutic effects of MSC-CM for stroke therapy. This study demonstrated that most preclinical studies of MSC-CM as a therapy for stroke recovery were based on rodent models of tMCAO. In addition, the current review indicated that MSC-CM has demonstrated ability to reduce infarct volume and improve motor function by targeting different cellular signaling pathways, promoting angiogenesis, neurogenesis and axonal growth and attenuating inflammation (Fig. 2).

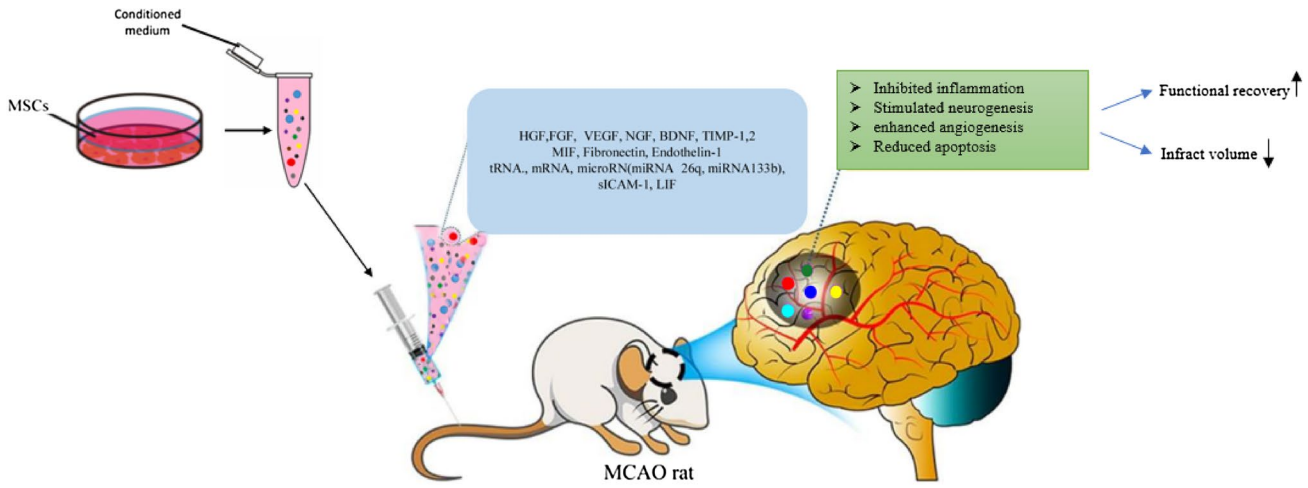

Fig. 2 Effects of conditioned medium derived from MSCs on stroke recovery

Ischemic cerebral stroke has been found to be a major cause of disability, death and morbidity [14]. Although endovascular therapy and rt-PA has been known as a gold standard treatment option, poor functional outcomes has been observed in approximately 40% of stroke survivors after treatment [13]. Many preclinical studies have demonstrated that MSCs are able to reduce cerebral ischemia reperfusion/ injury and improve functional outcomes [27, 53, 54]. Firstly, these neuroprotective effects were attributed to differentiation of grafted MSCs and replacement of dead neurons [55, 56]. Later, researchers found that neuroprotective effects of grafted MSCs are associated with promoting angiogenesis, neurogenesis and axonal growth through targeting different cellular pathways [36, 57]. Although some researchers have shown that stem cell therapy of ischemic therapy is safe, grafting cells might have some complications such as undesirable immune responses and tumor induction [23, 58]. To tackle the limitations of cell therapy of ischemic stroke, the researchers have focused on paracrine effects of stem cells [48]. It has been found that MSC-CM includes numerous growth factors, angiogenic factors, anti-apoptotic, and cytokines which play an important role in tissue regeneration [18, 46]. The current systematic review provided some crucial insights about treatment of ischemic stroke using MSC-CM: (I) MSC-CM has ability to improve motor recovery after an ischemic stroke by promoting angiogenesis and cellular signal pathways involved in this process [46], (II) MSC-CM is capable of reducing inflammation and apoptosis by attenuating microglia/macrophage infiltration and inhibiting Bax, Bcl2, caspase3, and pro-inflammatory cytokines [45, 47, 49], (III) MSC-CM has ability to improve BBB functional integrity and reduce its leakage following ischemic stroke in rats by targeting angiopoietin (Ang) 1 and tyrosine kinase (Tie) 2 [39, 42], (IV) In several previous studies by our group, we showed that neuroprotective effects of MSC-CM were associated with restoring mTOR activity, inhibiting acute autophagy, and promoting neurogenesis in an ERK1/2- BDNF pathway dependent mechanism [41, 52]. Additionally, the present systematic review provided another fundamental insight, indicating that the prepared MSC-CM under hypoxic conditions had stronger neuroprotective effects against ischemic stroke compared to the prepared MSC-CM under normoxic condition [40]. For instance, Jiang et al. demonstrated that the hypoxic CM group exhibited stronger effects for improvements in the mNSS and balance beam test in comparison with the DMEM and normoxic CM groups at Day 7 after MCAO [50]. Another important point is that the therapeutic time window can be considered a crucial factor when researchers use MSC-CM for stroke therapy. For example, Egashira et al. reported that MSC-CM administration 5 min after MCAO resulted in higher protective effects than administration 2 h after MCAO [44]. Moreover, it has been reported that MSC-CM administration in combination with other therapeutic agents can have better neuroprotective effects against ischemic stroke compared to monotherapy [51]. Asgari Taei showed that three times injections of CM are more effective than one time to create neuroprotective effects against ischemic stroke in a MCAO rat model [43].

Conclusion

Collectively, the current systematic review exhibits that MSC-CM can be considered a valid therapeutic agent for improving motor function and reducing injury in animal model of ischemic stroke. Although preclinical studies demonstrate reducing infarct volume, there are no clinical trials in stroke patients and hence its therapeutic efficacy and safety is unknown in humans. We expect that current

systematic review provides greater insights on the potential use of MSC-CM for ischemic stroke therapy.

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All protocols were approved by Ethical Committee of Dezful University of Medical Sciences, Dezful, Iran.

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