



COVID-19-Induced Stroke and the Potential of Using Mesenchymal Stem Cells-Derived Extracellular Vesicles in the Regulation of Neuroinflammation

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Abstract

Ischemic stroke (IS) is a known neurological complication of COVID-19 infection, which is associated with high mortality and disability. Following IS, secondary neuroinflammation that occurs can play both harmful and beneficial roles and lead to further injury or repair of damaged neuronal tissue, respectively. Since inflammation plays a pivotal role in the pathogenesis of COVID-19-induced stroke, targeting neuroinflammation could be an effective strategy for modulating the immune responses following ischemic events. Numerous investigations have indicated that the application of mesenchymal stem cells-derived extracellular vesicles (MSC-EVs) improves functional recovery following stroke, mainly through reducing neuroinflammation as well as promoting neurogenesis and angiogenesis. Therefore, MSC-EVs can be applied for the regulation of SARS-CoV-2-mediated inflammation and the management of COVID-19-related ischemic events. In this study, we have first described the advantages and disadvantages of neuroinflammation in the pathological evolution after IS and summarized the characteristics of neuroinflammation in COVID-19-related stroke. Then, we have discussed the potential benefit of MSC-EVs in the regulation of inflammatory responses after COVID-19-induced ischemic events.

Keywords COVID-19 · Stroke · Neuroinflammation · Mesenchymal stem cells · Extracellular vesicles · Exosome

Introduction

Stroke is the second leading cause of mortality worldwide and the most common cause of long-term disability in Western countries (Rajkovic et al. 2018; Jayaraj et al. 2019). This neurological disorder occurs following the acute injury of the brain by a vascular reason (Campbell and Khatri 2020). Ischemic stroke (IS) is the most common type of stroke due to the reduced blood flow to a certain part of the brain and accounts for about 85% of all acute strokes (Tadi and Lui 2020). IS leads to energy depletion and triggers excitotoxicity and neuroinflammation in the vicinity of brain injury (Liu et al. 2017).

Several disorders, such as hematologic diseases, cardiovascular abnormalities, and infection increase the risk of IS (Woldu 2020; Ferro and Infante 2021; Virani et al. 2021). Coronavirus disease 2019 (COVID-19) results from SARS-CoV-2 is an inflammatory disorder (Leisman et al. 2020; Gorji and Khaleghi Ghadiri 2021), which can affect the central nervous system (CNS) (Soltani Zangbar et al. 2021; Rezaeitalab et al. 2021). COVID-19 predisposes

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some patients to a wide range of neurological complications, including stroke (Ntaios et al. 2020). It is estimated that stroke occurs in about 2–6% of the hospitalized patients with COVID-19 (Ellul et al. 2020). It has been shown that SARS-CoV-2 is more likely to induce thrombotic complications, such as IS compared to influenza infection (Merkler et al. 2020). Although COVID-19-induced stroke is more common in elderly people with underlying disorders, such as hypertension, atherosclerosis, or atrial fibrillation, it is mainly linked to a hypercoagulable state caused by systemic inflammation and cytokine storm (Spence et al. 2020; Fifi and Mocco 2020). Aside from the neuroinflammation induced by systemic immune activation, direct invasion of SARS-CoV-2 through binding its spike protein to the receptor angiotensin-converting enzyme 2 (ACE2) may lead to neuroinflammation and cytokine release (Sriwastava et al. 2021; Song et al. 2021).

Numerous investigations have indicated that the application of MSC-EVs improves functional recovery following stroke, mainly through reducing neuroinflammation as well

as promoting neurogenesis and angiogenesis (Jiang et al. 2018; Deng et al. 2019; Yang et al. 2020). This review aimed to summarize the features of neuroinflammation induced by COVID-19 and non-COVID-19 stroke and then discuss therapeutic effects and underlying mechanisms of action of MSCs-EVs in the modulation of neuroinflammation and improvement of functional recovery following stroke.

Neuroinflammation in the Pathological Evolution After IS

Neuroinflammation is a major and primary pathological event involved in IS (Fig. 1) (Lian et al. 2021). Indeed, neuroinflammation is a common feature in a wide variety of CNS diseases, such as IS (Stephenson et al. 2018). Within the CNS, an inflammatory response is considered as the capability of the neuronal tissues to drive neuroprotection against pathological conditions (Mendiola and Cardona 2018). The initiation and development of neuroinflammation

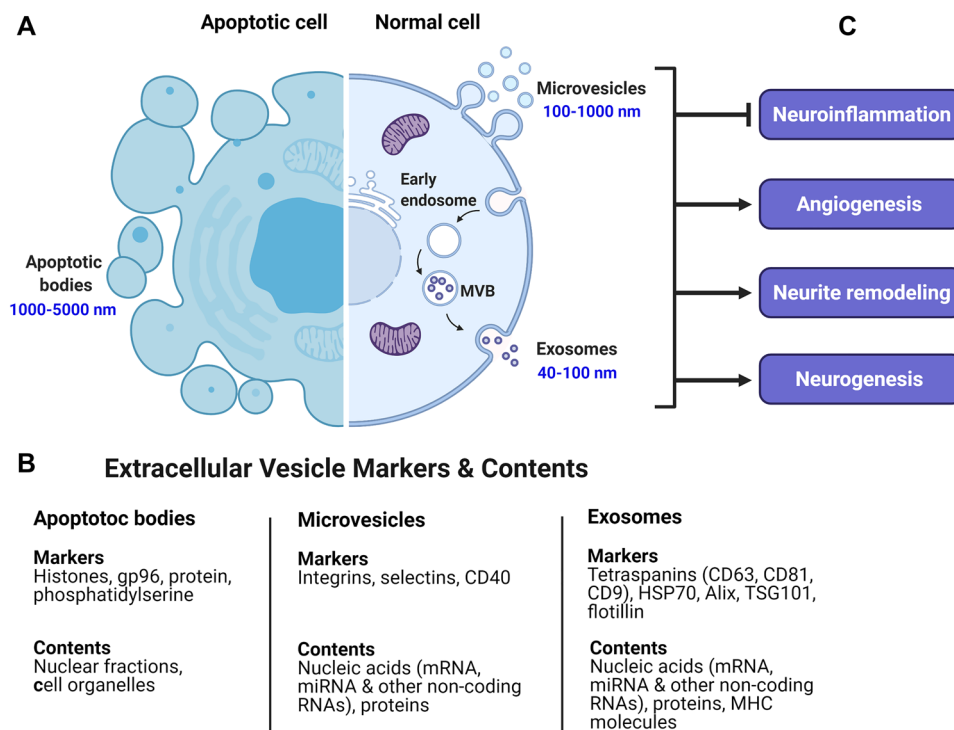


Fig. 1 Schematic overview of the general mechanisms of neuroinflammation after ischemic stroke. Ischemic stroke occurs following a blood vessel occlusion due to a thrombus or embolus. Brain ischemia results in oxygen and ATP depletion and subsequently acute neuronal damage in the infarcted tissue. Then, danger-associated molecular patterns (DAMPs) are released by injured and dying neurons which in turn activate microglia, astrocytes, and endothelial cells, leading to the produce the proinflammatory factors such as IL-1, IL-6 and TNF, reactive oxygen species (ROS), matrix metalloproteinases (MMPs), and vascular endothelial growth factor (VEGF). These molecules

trigger BBB dysfunction by endothelial downregulation of tight junction proteins and upregulation of cell adhesion molecules including vascular cell adhesion molecules (VCAM) and intracellular adhesion molecule-1 (ICAM-1), leading to migration and infiltration of circulating leukocytes, mainly neutrophils through the disrupted BBB into the ischemia area. Activated neutrophils release proinflammatory mediators which cause further activation of microglia and astrocytes. This pathological cascade leads to neuronal cell death, edema, and hemorrhage, which in turn cause further neuroinflammation and tissue damage

presumably are based on the interaction between glial cells, neurons, and immune cells (Skaper et al. 2017). Glial cells, in particular microglia, present a central role in the initiation of neuroinflammation via producing proinflammatory mediators (Almeida et al. 2020). In homeostatic conditions, neurons actively induce anti-inflammatory signals and subsequently prevent microglia polarization to the inflammatory M1 phenotype (Bernaus et al. 2020). In response to various stresses such as IS, microglial cells are rapidly activated and polarized into the proinflammatory M1 or the anti-inflammatory M2 phenotypes, which are involved in secondary brain damage or repair, respectively (Jiang et al. 2020).

Microglia activation is the hallmark of neuroinflammation and occurs immediately following stroke (He et al. 2020). The M1 polarized microglia are functionally characterized by the capability of eliminating microorganisms or tumor cells (Lanza et al. 2021). They also release proinflammatory cytokines (IL-1 β , IL-6, IL-12, IFN- γ , and TNF), chemokines (CCL-2, CCL-20), CXCL-10, and cytotoxic substances, including reactive oxygen species (ROS), reactive nitrogen species, excitatory amino acids, and prostaglandins E2 (Liu et al. 2019). These proinflammatory factors, particularly inducible nitric oxide synthase, have cytotoxic effects on neurons, leading to neuronal loss, disruption of the blood–brain barrier (BBB) and the degradation of the extracellular matrix (ECM) (Zhang et al. 2021a, b). Alternatively, M2 microglia induces phagocytosis of dead neurons stronger and is associated with neural survival, reduction of brain damage, and restriction of destructive immune response (Lanza et al. 2021; Zhang et al. 2021a, b). M2 phenotype is activated to exert a beneficial role following IS by producing anti-inflammatory and neuroprotective mediators including, IL-10, IL-4, and TGF- β (Jiang et al. 2020). Among these, IL-4 is an important cytokine involved in the improvement of functional recovery after IS (Zheng and Wong Division 2019).

The function and nature of neuroinflammation can be either supportive or destructive based on the conditions and the intensity as well as the duration of inflammation (Liu et al. 2017). Evidence indicates that the proinflammatory state is probably dominant in the acute phase of IS (Jiang et al. 2020). Although the production of transient proinflammatory mediators, particularly cytokines, in the early and acute stages of brain injuries plays a neuroprotective role, including promoting injury recovery and axonal re-growth (Liu et al. 2017), long-term and supra-physiological production of inflammatory mediators following uncontrolled and persistent glial activation causes chronic inflammation and leads to secondary damage (Bronzuoli et al. 2016). The pro-inflammatory mediators are detrimental to the hypoxic tissues. Glial cells can damage healthy neurons and lead to decreased neuronal plasticity and cognitive impairments (Liu et al. 2017;

Jurga et al. 2020). Therefore, the imbalance between M1 and M2 polarization states and shifting to the M1 phenotype contributes to the pathogenesis of neuroinflammation following brain injury events such as IS (Liu et al. 2019). Thus, modulation of the microglial phenotype is one of the most important strategies to reduce inflammatory responses and promotion of brain repair, via reducing brain edema, improving the integrity of white matter, promoting neurogenesis, and recovering motor function (Liu et al. 2019; Jiang et al. 2020). However, the sustained inhibition of microglia at the early stages of stroke interferes with neurogenesis (Xiong et al. 2016). Therefore, a desirable anti-inflammatory agent for alleviating IS should maintain the physiological activation of glial cells (Bronzuoli et al. 2016).

Both in vitro and in vivo studies have highlighted the crosstalk of microglia with astrocytes in the neurovascular unit (Bernaus et al. 2020). These studies have indicated that microglia are more sensitive to damage and activated in the acute phase of IS. Then, the activated microglia produce signaling molecules, such as TNF, IL-1, and IL-1 receptor antagonist (IL-1Ra) and trigger reactive astrocytes (Liu et al. 2020a, b). Astrocytes use the same neuroprotective and immunoregulatory mechanisms for both the maintenance of homeostasis and response to injury (Cekanaviciute and Buckwalter 2016). Similar to microglia, astrocytes release a variety of pro and anti-inflammatory cytokines and chemokines under different conditions (Whitney et al. 2009). Astrocytes are known as the important in situ regulators of the neuroinflammatory processes in stroke and gain the reactive phenotype in the subacute time frame of 2 days to 1 week after stroke, which is associated with the expression of cytokines and peak inflammatory response (Cekanaviciute and Buckwalter 2016).

The reactive astrocytes regulate immune responses by infiltration and activation of immune cells, which motivate macrophages to acquire either proinflammatory or anti-inflammatory phenotypes. They exert both harmful (A1 reactive astrocytes) and beneficial (A2 reactive astrocytes) effects on neuroinflammation and neurological outcomes following brain injury (Cekanaviciute and Buckwalter 2016). Evidence indicated that the kind of stimuli during neuroinflammation may switch the function of astrocytes from beneficial to detrimental (Colombo and Farina 2016). The prolonged expression of proinflammatory mediators, such as TNF, IL-12, IL-17, CXCL10, intensify the infiltration of T lymphocytes and myeloid cells into the CNS and may result in uncontrolled chronic inflammation (Cekanaviciute and Buckwalter 2016). It may be helpful to moderately weaken the activation and shorten the M1/A1 or strengthen the A2/M2 timeline for better brain repair and functional recovery after IS (Liu et al. 2020a, b).

IS and Neuroinflammation in COVID-19 Patients

Ischemic stroke is known as a presenting feature of COVID-19 and is associated with a poor prognosis and high mortality in patients with COVID-19 (Shah et al. 2020; Zhang et al. 2021a, b). Although the stroke in patients with COVID-19 could be due to aging and underlying comorbidities, such as hypertension, diabetes, malignancy, and cardiovascular disorders, COVID-19-induced stroke is mainly linked to a hypercoagulable state caused by systemic inflammation and is termed sepsis-induced coagulopathy (Spence et al. 2020; Fif and Mocco 2020; Jarrahi et al. 2020). Indeed, blood coagulation plays a fundamental role in COVID-19-mediated stroke (Yaghi et al. 2020).

In addition to triggering a hypercoagulable state, a direct viral infection of endothelial cells can result in endotheliitis and eventually endothelial dysfunction (Nannoni et al. 2021). Inflammation and apoptosis of endothelium following infection by SARS-CoV-2 have been reported at autopsy samples from lung, heart, kidney, and bowel (Pezzini and Padovani 2020). Similar to these organs, SARS-CoV-2 can directly infect BBB endothelium through binding to ACE-2 receptor and increase the risk of viral-induced vasculitis, BBB injury, and subsequently IS as the long-term outcome for patients (Berger 2020; Bodro et al. 2021). Moreover, infection of endothelial cells can be followed by perivascular astrocytes and macrophages due to proximity with infected endothelium. Subsequently, viral infection may be transmitted to microglia and neurons (Murta et al. 2020). On the other hand, BBB breakdown as an outcome of systemic inflammation and cytokine storm can facilitate SARS-CoV-2 penetration to the CNS. Excessive levels of proinflammatory cytokines, particularly IL-6, TNF, MIP-1 α , CXCL10 (IP-10), G-CSF, C-reactive protein, and ferritin can destabilize the integrity of tight junctions between BBB endothelial cells, thus enabling viral entry (Alam et al. 2020). Subsequently, microglia and astrocytes as part of local neuroinflammatory responses, express pattern recognition receptors (PRRs), to identify and interact with pathogen-associated molecular patterns (PAMP) required initiating and/or augmenting innate immunity within the CNS (Murta et al. 2020).

All these pathways result in astrocytes and microglia infection (as the viral hosts) or their hyperactivation after receiving proinflammatory signals arise from systemic inflammation, which finally leads to shifting to a proinflammatory state and expanding neuroinflammation (Murta et al. 2020). Moreover, evidence has indicated that invasion of SARS-CoV-2 through the olfactory system into the brain parenchyma can drive neuroinflammation (De

Melo et al. 2020). Furthermore, SARS-CoV-2 may reach the CNS through other peripheral nerves including the vagus nerve, which enables the virus to access the brain stem from the lungs and gut (the lung-gut-brain axis) (Chigr et al. 2020; Zhang et al. 2020). This may explain a combination of the lung, gastrointestinal, and neurological involvement during infection with COVID-19 in some patients (Alam et al. 2020). Furthermore, there is a less common route for penetration and spreading of the virus through the lymphatic drainage system (Finsterer and Stollberger 2020). Eventually, all these events are associated with the increased risk of ischemic or hemorrhagic stroke and neuroinflammation (Bodro et al. 2021).

The Potential Use of MSC-Derived EVs in Modulating Neuroinflammation Following COVID-19-Induced Stroke

Currently, there is no specific and effective antiviral treatment available for COVID-19 patients, and countless clinical investigations are trying to find therapeutic approaches for different forms of the disease. Physicians and researchers are applying and evaluating the effect of mesenchymal stromal/stem cells (MSCs) or their secretomes, including EVs, in patients with COVID-19. These ongoing studies will provide significant information for the treatment of COVID-19 or the prevention of severe consequences of the disease (Alzahran et al. 2020; Pocsfalvi et al. 2020).

MSC-EVs emulate the immunoregulatory characteristics and the regenerative function of MSCs (Fig. 2) (Wiklander et al. 2019). Several studies have shown that intravenous or intratracheal administration of MSC-exosome (Exos) attenuates acute lung injury through decreased levels of inflammatory mediators as well as monocyte infiltration into the lung and subsequently protects alveolar epithelia during acute respiratory distress syndrome (ARDS) in the patients with COVID-19 (Tsuchiya et al. 2020; Rezakhani et al. 2020). MSC-Exos suppress cytokine storm via inhibiting the proinflammatory factors IL-1 β , IL-6, IL-17, TNF, and IFN- γ , and promote M2 phenotype for releasing anti-inflammatory factors, such as IL-4, IL-10, and TGF- β (Jayaramayya et al. 2020). These protective effects of MSC-Exos may be linked to exosomal active mitochondria, which promote the switching of macrophage polarization from M1 to M2 state via the enhancement of oxidative phosphorylation (Al-Khawaga and Abdelalim 2020).

Evidence has emphasized that MSCs-EVs exert a considerable role in neuroinflammation, neurogenesis, and neurogenic niches, and thus can be considered as a promising therapeutic strategy in neurological diseases such as stroke. Indeed, MSCs-EVs reduce neuroinflammation through modulating proinflammatory responses and induce neurogenesis

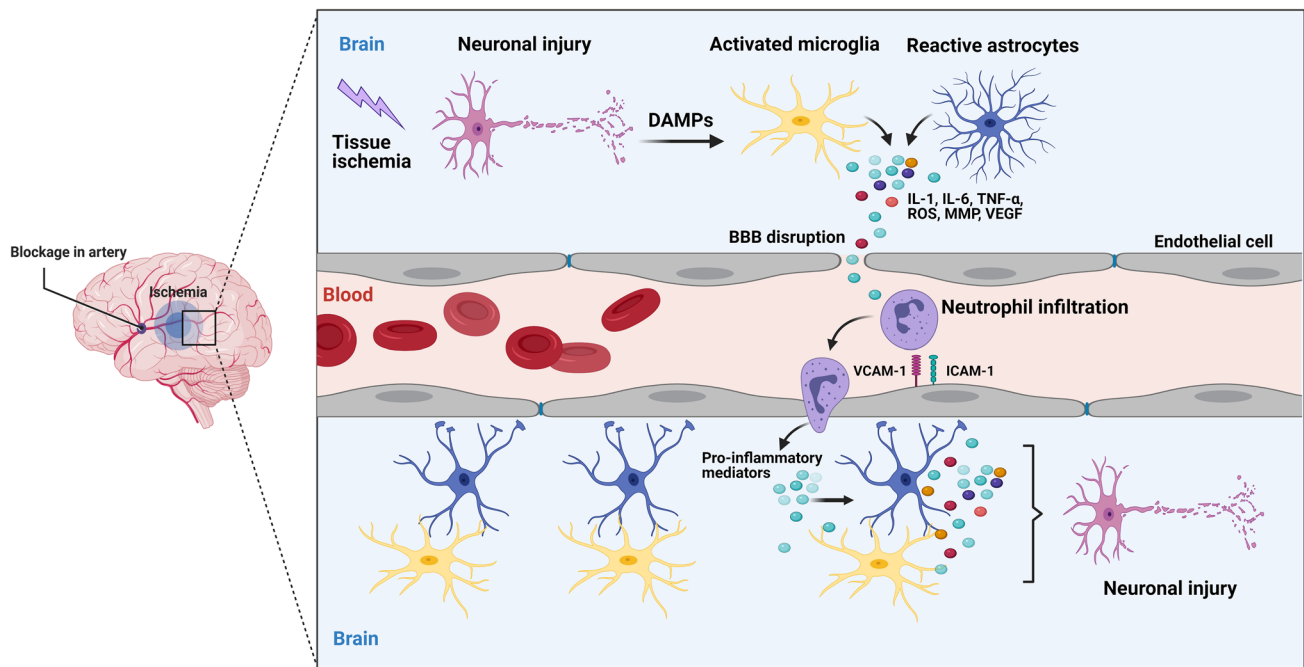


Fig. 2 Formation and composition of extracellular vesicles (EVs) and their therapeutic effects in reducing brain inflammation. **A** EVs are routinely categorized based upon their biogenesis, size, surface markers, contents and functions, and include exosomes, microvesicles, and apoptotic bodies. Exosomes are produced in the endosomal compartment by inward budding of plasma membrane and formation of early endosomes. Subsequently, early endosomes mature into late endosomal multivesicular bodies (MVBs) which contain membrane-bound intraluminal vesicles. Then intraluminal vesicles, i.e., exosomes release into the extracellular milieu upon fusion of MVBs with the cell's plasma membrane through exocytosis. Microvesicles arise as a

result of direct outward budding of the plasma membrane into extracellular space (blebbing from living cells), and apoptotic bodies are only formed by cells undergoing apoptosis (blebbing from apoptotic cells). **B** Different EVs contain various markers and cargoes including proteins, lipids, and nucleic acids. **C** MSC-EVs, including Exosomes and microvesicles, attenuate neuroinflammation mainly through suppressing the activation of astrocytes and microglia and shifting to an anti-inflammatory state. Moreover, MSC-EVs promote angiogenesis and neurite remodeling as well as neurogenesis possibly by inducing the neurogenic niche

and angiogenesis (Yang et al. 2017), subsequently promoting functional recovery (Table 1). Recently, the results of a systematic review showed that the beneficial effects of MSC-Exos therapy in animal models of stroke were linked to inhibition of inflammation and oxidative stress as well as enhanced angiogenesis, neurogenesis, and neurite remodeling (Dehghani et al. 2020). Pathipati et al. stated that exosomes derived from bone marrow MSCs (BMSC-Exos) are taken up by microglial cells isolated from the injured brain regions of neonatal rats following IS and subsequently glia activation and inflammatory responses are suppressed (2019). One of the most important mechanisms involved in the therapeutic properties of MSC-EVs applied in brain ischemia is the transportation of miRNAs between cells. MSC-EVs contain miRNAs that contribute to neurological functions in animal models of IS through regulation of post-transcriptional gene expression in target cells (Dabrowska et al. 2019a, b).

Moreover, MSC-EVs induce endogenous brain cells to release exosomal miRNAs and improve brain plasticity following IS (Chen and Chopp 2018). Huang et al., for

instance, have demonstrated that the exosomal miR-124-3p derived from microglia stimulates their polarity towards an anti-inflammatory state while inhibiting proinflammatory responses, eventually triggering the repair of damaged neurons. This evidence suggests that upregulating miRNAs such as miR-124-3p in MSC-EVs could be considered as a therapeutic approach for targeting neuroinflammation and recovering the damaged neurons following stroke (2018). In another study, adipose MSC-derived exosomes (ADMSC-Exos) enriched with miR-30d-5p have displayed the protective effects in patients with IS as well as in animal models of stroke. Exosomes derived from microRNA-30d-5p-overexpressing ADMSC mostly attenuated brain injury during the acute phase of stroke by suppressing autophagy and stimulating M2 microglial polarization (Jiang et al. 2018). Moreover, Deng et al. have reported that BMSCs deliver miR-138-5p to the astrocytes by exosomes and remarkably suppress the injury volume and inflammation, presumably via the regulation of the expression of the neutrophil gelatinase-associated lipocalin (LCN2) in an IS mouse model (2019).

Table 1 The efficacies of MSC-derived EVs on neuroinflammation

Source of exosome	Disease model	Clinical value	Bioactive components	Ref
BMSC	Rat model of TBI	BMSC-Exos increased brain angiogenesis and neurogenesis, and reduced neuroinflammation	NA	(Zhang et al. 2015)
BMSC	Rat model of TBI	BMSC-Exos did not reduce lesion size but remarkably improved spatial learning, sensorimotor functional recovery. Exosome treatment increased neurogenesis, and reduced neuroinflammation. BCMSC-Exos cultured in 3D scaffolds exerted better outcome in spatial learning compared with BCMSC-Exos cultured in the 2D condition	NA	(Zhang et al. 2017)
BMSC	Mouse model of TBI	Administration of the BMSC-EVs decreased the levels of proinflammatory cytokine IL-1 β in a dose-dependent manner and suppressed neuroinflammation after TBI	NA	(Kim et al. 2016)
ADMSC	Patient and rat model of IS	microRNA-30d-5p-overexpressing ADMSC attenuated brain injury during the acute phase of stroke by suppressing autophagy and stimulating M2 microglial polarization	microRNA-30d-5p	(Jiang et al. 2018)
BMSC	Mouse model of IS	microRNA-138-5p-overexpressing BMSC suppressed the injury volume and neuroinflammation presumably via the regulation of the lipocalin 2 (LCN2) expression	microRNA-138-5p	(Deng et al. 2019)
UCMSC	Rat model of IS	UCMSC-Exos and specially exosomes derived from CCR2-overexpressing UCMSCs exhibited beneficial effects on oligodendrogenesis, remyelination, and polarization, and improved cognitive functions following stroke. Exos ^{CCR2} inhibited the activation of macrophages and suppressed M1 polarization of microglia	CCR2	(Yang et al. 2020)
BMSC	Rat model of IS	BMSC-Exos in combination with rosuvastatin improved the functional recovery, promoted neuroprotection, and reduced neuroinflammation and cell death	NA	(Safakheil 2020)
ADMSC	Rat model of IS	Exosomes derived from miRNA-126-overexpressing ADMSCs promoted functional recovery by improving neurogenesis and suppressing neuroinflammation	miRNA-126	(Geng et al. 2019)
ADMSC	Rat model of IS	The combination of ADMSCs and ADMSC-Exos significantly suppressed production of ROS and oxidative stress and subsequently inflammation in the setting of ischemia–reperfusion injury in AIS animals	NA	(Chen et al. 2016)
BMSC	Mouse model of ALS	BMSC-EVs primed with IFN- γ suppressed neuroinflammation via specific immunomodulatory miRNAs acting on microglia more effective than unprimed BMSCs	miR-467f miR-466q	(Giunti et al. 2021)

Table 1 (continued)

Source of exosome	Disease model	Clinical value	Bioactive components	Ref
BMSC	Mouse model of SCI	BMSC-EVs under hypoxic preconditioning promoted functional recovery and suppressed neuroinflammation following spinal cord injury by shifting microglial M1/M2 polarization	miR-216a-5p	(Liu et al. 2020a, b)
WJMSC	Rat model of perinatal brain injury	Intranasally administration of WJMSC-EXos reduced microglia-mediated neuroinflammation in rats with perinatal brain injury	NA	(Thomi et al. 2019)

BMSC bone marrow MSC, ADMSC adipose MSC, UCMSC umbilical cord MSC; WJMSC Wharton's jelly MSC, AIS acute ischemic stroke, ALS amyotrophic lateral sclerosis, SCI spinal cord injury, NA not available

Recently, researchers have evaluated the effects of exosomes derived from umbilical cord MSCs (UCMSC-Exos) and also exosomes derived from CCR2-overexpressing UCMSCs on post-stroke cognitive impairment. Both naive and enriched UCMSC-Exos exhibited beneficial effects on oligodendrogenesis, remyelination, and polarization, and improved cognitive functions following stroke. However, exosomes enriched by CCR2 (Exos^{CCR2}) showed remarkable efficiency compared to naive exosomes (Yang et al. 2020). CCL2 is highly expressed in the injured tissues and triggers the activation and recruitment of resident microglia in the ischemic injury site (Tian et al. 2017; Li et al. 2020). Moreover, CCL2 promotes infiltration of CCR2-expressing cells, including peripheral monocytes, T lymphocytes, and natural killer cells, to the injured parenchyma (Semple et al. 2010). Exos^{CCR2} through binding to CCL2 inhibited the infiltration and activation of macrophages and suppressed M1 polarization of microglia/macrophages (Yang et al. 2020).

Evidence indicated that the delivery route of MSC-EVs can also impact their therapeutic efficiency. In contrast to MSCs, intravenously administration of EVs cannot reduce stroke-induced neuroinflammation. However, intra-arterially injection of BMSC-EVs alleviates ischemia-induced glial cell activation and infiltration of T cytotoxic lymphocytes and significantly decreases the proinflammatory cytokines and chemokines in a rat model of IS (Dabrowska et al., 2019a, b). Since stroke is a common and devastating complication during SARS-CoV-2 infection and given the promising results of using stem cells or their secretomes in early studies as well as confirmed positive effects of using MSC-EVs for reducing neuroinflammation in animal models of stroke, MSC-EVs can apply at least as the supportive treatment in COVID-19 patients for targeting primary or secondary neuroinflammation induced by direct CNS infection or cytokine storm, respectively (Sriwastava et al. 2021; Amruta et al. 2021; Dabrowska et al. 2021).

Conclusion and Future Prospects

MSC-EVs are emerging therapeutics for a wide range of inflammatory and degenerative disorders, and are at the beginning of the path to translate from preclinical studies into clinical trials (Forsberg et al. 2020). MSC-EVs possess many advantages compared to classical cell therapy. Higher immunomodulatory effects of MSC-EVs with low immunogenicity, the ability to scalable production and storage of EVs for further usage, make them as promising therapeutic tool to treat neurodegenerative disorders, such as COVID-19-induced stroke, in which neuroinflammation plays a determining role in the patient's fate (Schultz et al. 2021; Giunti et al. 2021).

It is claimed that MSC-EVs exert superior rehabilitation effects than MSC therapy in stroke conditions which may be due to the higher capacity of EVs to cross the BBB and thus reach ischemic regions faster compared with MSCs (Moon et al. 2019). Most preclinical studies indicated that either naive or engineered MSC-EVs exert influential therapeutic effects in stroke and neurological injury (Zhang et al. 2019). The results of these studies have demonstrated the impact of MSC-EVs in improving functional recovery, increased neurogenesis as well as reduced neuroinflammation. Although, MSC-EVs can act as anti-inflammatory agents to modulate inflammatory conditions, however, another advantage of MSC-EVs is the possibility to payload with other therapeutic agents (O'Driscoll 2020). For instance, in terms of the COVID-19, antiviral-loaded EVs can be administrated intranasally or by inhalation to target the nasal mucosa and the lungs, as two sites where the coronavirus is frequently localized (Popowski et al. 2021). Moreover, EVs have the potential to be modified by exerting molecular engineering approaches to optimize desired functions. For example, genetically modified MSC-EVs enriched by miRNA-124 are assessed in acute IS patients in phase 1–2 clinical trials (Öztürk et al. 2021).

However, the clinical use of MSC-EVs is still debatable due to the complexity of MSCs given the tissue origin and also cell culture conditions (Wiklander et al. 2019). In addition, EVs heterogeneity, isolation protocols and subsequently yield and purity, detection and characterization methods, delivery routes, effective dosages, application times, and end-points have remained a controversial challenge. Therefore, biomanufacturing standards and standardized criteria need to be considered for the therapeutic application of MSC-EVs, particularly to treat severe and inflammatory conditions such as COVID-19-induced stroke (Forsberg et al. 2020). For instance, since the sustained inflammatory response and hypercoagulability are considered as the hallmark or consequence of infection with SARS-CoV-2, it is proposed that procoagulant factors and proinflammatory cytokines present in the EVs cargo must be removed before administration (Lee et al. 2021). Moreover, estimating the optimal efficient dose of MSC-EVs is one of the criteria which must be considered. Recently, Otero-Ortega et al. evaluated various doses of EVs including 50, 100, or 200 µg intravenously in rat models of stroke. They reported 50 µg of MSC-EVs can effectively promote brain protection, repair, and recovery following a subcortical IS. Nonetheless, the dosages lower than 50 µg of EVs were not sufficient and effective (2020).

Despite the favorable outcomes in small animal models, well-established non-human primate models are required to evaluate the safety and efficacy of MSC-EVs in non-COVID and COVID-19-induced stroke. Furthermore, well-controlled and rationally designed clinical studies using naïve MSC-EVs derived from different tissue as well as genetically engineered MSC-EVs are needed to investigate their efficiency and adverse effects.

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent This article does not contain any studies with human participants so it is not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

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