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Review

Therapeutic potential of stem cells in subarachnoid hemorrhage

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Abstract

Aneurysm rupture can result in subarachnoid hemorrhage, a condition with potentially severe consequences, such as disability and death. In the acute stage, early brain injury manifests as intracranial pressure elevation, global cerebral ischemia, acute hydrocephalus, and direct blood–brain contact due to aneurysm rupture. This may subsequently cause delayed cerebral infarction, often with cerebral vasospasm, significantly affecting patient outcomes. Chronic complications such as brain volume loss and chronic hydrocephalus can further impact outcomes. Investigating the mechanisms of subarachnoid hemorrhage-induced brain injury is paramount for identifying effective treatments. Stem cell therapy, with its multipotent differentiation capacity and anti-inflammatory effects, has emerged as a promising approach for treating previously deemed incurable conditions. This review focuses on the potential application of stem cells in subarachnoid hemorrhage pathology and explores their role in neurogenesis and as a therapeutic intervention in preclinical and clinical subarachnoid hemorrhage studies.

Key Words: delayed cerebral ischemia; early brain injury; matricellular protein; neurogenesis; stem cell therapy; subarachnoid hemorrhage

Introduction

Subarachnoid hemorrhage

Epidemiology

The rupture of cerebral aneurysms leads to subarachnoid hemorrhage (SAH), contributing to significant mortality rates despite ongoing preventive efforts (Suarez et al., 2006; Kanamaru et al., 2020; Hoh et al., 2023). Risk factors for SAH, such as hypertension, smoking, alcohol consumption, and the presence of unruptured cerebral aneurysms with a high rupture risk, have been extensively studied (Hoh et al., 2023). Despite efforts to mitigate these risks, SAH continues to affect 2%–5% of all strokes, with an annual incidence of 10.5 cases per 100,000 population worldwide, significantly impacting the lives of working-age adults (Suarez et al., 2006; Kanamaru et al., 2020). Major predictors of poor SAH outcomes include initial neurological severity, rebleeding, older age, cerebral vasospasm, delayed cerebral infarction, and systemic complications (Kanamaru et al., 2020; Hoh et al., 2023). Treatment strategies for SAH typically involve controlling blood pressure to prevent rebleeding, with emergency external ventricular drainage performed in cases of acute hydrocephalus, followed by aneurysm repair (Neifert et al., 2021). Post-repair, SAH patients are often managed in neuro-intensive care units to monitor cerebral vasospasm, delayed cerebral infarction, and systemic complications (Hoh et al., 2023). Mortality rates prior to hospital admission for SAH have been documented at 22%–26% (Korja et al., 2016). Despite the absence of improvement in hospital inpatient mortality rates for SAH, which remained at 13.7% in 2006 and declined slightly to 13.1% in 2018 in the United States and ranged from 19% to 20% globally in 2021, studies based on population data indicate an overall reduction in case-fatality rates by 1.5% per year between 1980 and 2020, albeit with considerable variation among different countries (Hoh et al., 2023). In 2020, age-adjusted mortality rates for SAH were highest in Oceania, Andean Latin America, and Central Asia (Tsao et al., 2023).

Pathologies in the acute phase after SAH: early brain injury and delayed cerebral ischemia

Patients with SAH may develop new symptoms within 14 days, even after successful aneurysm repair, mainly due to cerebral vasospasm (Vergouwen et al., 2010). Clinically, delayed neurological deficits are known as delayed cerebral ischemia, characterized by new neurological deficits or impaired consciousness lasting at least one hour, with no other obvious cause (e.g., epilepsy, meningitis, and metabolic imbalance) (Vergouwen et al., 2010). While cerebral vasospasm was previously thought to be the main cause of delayed infarction, recent studies suggest that delayed infarction is not always caused by cerebral vasospasm (Suzuki et al., 2020). Instead, the concept of early brain injury (EBI) has emerged, indicating that a series of pathological changes occur after aneurysm rupture (Kanamaru and Suzuki, 2019; Suzuki et al., 2020). EBI starts with bleeding from an aneurysm, damaging surrounding brain tissue and leading to elevated intracranial pressure, global cerebral ischemia, acute hydrocephalus,

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and the release of blood contents like fibrinogen, heme, thrombin, and leukocytes (Kanamaru and Suzuki, 2019). These factors can directly affect brain tissue or dissolve in cerebrospinal fluid (CSF), potentially causing brain insult and delayed cerebral infarction, which significantly impacts patient outcome (Kanamaru and Suzuki, 2019; Suzuki et al., 2019, 2020). Pathological changes such as inflammation, neuronal apoptosis, blood–brain barrier (BBB) disruption, hypoxia, microthrombosis, early vasospasm, impaired cerebral blood flow autoregulation, venous drainage dysfunction, metabolic imbalance, and cortical spreading depolarization are proposed factors contributing to EBI and delayed cerebral ischemia (Suzuki et al., 2021).

In early clinical trials aimed at improving SAH patient outcomes, reducing cerebral vasospasm was the primary focus. Allen et al. (1983) conducted a randomized-controlled trial to assess whether the calcium channel blocker nimodipine could prevent neurological deficits from cerebral vasospasm. They found that nimodipine significantly reduced the occurrence of new neurological deficits or death. Currently, nimodipine is the only recommended drug for managing cerebral vasospasm with a class 1 recommendation and level A evidence, according to the American Heart Association and American Stroke Association guidelines (Hoh et al., 2023). Subsequent to this study, numerous clinical trials were conducted (Allen et al., 1983; Haley et al., 1995; Siironen et al., 2003; van den Bergh et al., 2006; Gomis et al., 2010; Macdonald et al., 2011; Qureshi et al., 2021); however, highly effective treatments for EBI have yet to be established (Suzuki and Nakano, 2018). Thus, the search for targeted treatments for EBI/delayed cerebral ischemia remains essential.

Pathologies in the chronic phase after SAH

Even if patients survive the acute phase of SAH, they may still experience persistent symptoms. Up to 33% of survivors are unable to return to work (Hackett and Anderson, 2000). A meta-analysis revealed that from six studies, 55% of SAH patients were independent, 19% were dependent, and the rest died within 1–12 months of follow-up (Nieuwkamp et al., 2009). In the chronic phase, various degrees of changes in brain structure, including grey matter (Bendel et al., 2010), white matter (Reijmer et al., 2018), and pituitary gland (Rass et al., 2020) can be observed after SAH, which may be related to neurological impairment (Stehouwer et al., 2018). Iron deposition may also occur, predominantly observed in white matter by magnetic resonance imaging, and may result in volume loss, relating to cognitive dysfunction in patients with good clinical grade SAH (Scherfler et al., 2016). In postmortem brain tissue from SAH patients, iron deposition was observed most on the brain surface and decreased with distance from the surface (Galea et al., 2022). Extravasated blood and the breakdown of red blood cells result in a higher concentration of hemoglobin in the subarachnoid space, which may cause further iron deposition and relate to cognitive deficits (Galea et al., 2022). Iron chelation to prevent iron deposition in the brain has been shown to be neuroprotective in SAH pathology. For example, deferoxamine reduced the number of microvasospasms (Liu et al., 2021a), and improved cognitive status (LeBlanc et al., 2016) in preclinical studies; however, currently, this is not conclusive for translation to the clinical setting.

One major complication in the chronic phase is the development of chronic hydrocephalus, which may be caused by alterations in CSF flow, obstruction of arachnoid granulations by blood products, and adhesion of the ventricular system (Kuo and Huang, 2021). An important pathological change in chronic hydrocephalus is fibrosis of the leptomeninges and arachnoid granulations, which affects CSF flow and can further develop hydrocephalus (Kuo and Huang, 2021). Transforming growth factor (TGF)-β, a fibrogenic factor, has been reported to be produced externally by extravasated blood, and TGF-β itself acts as a chemoattractant for inflammatory cells, promoting endogenous TGF-β production further (Yan et al., 2016). From these factors in the chronic phase of SAH, occurrences of brain volume loss due to various pathologies of brain injury (including cell death) and fibrosis of leptomeninges and arachnoid granulations could occur and affect patients' prognosis. Patients may receive rehabilitation for varying levels of disabilities, and in cases of chronic hydrocephalus, shunt surgery might be an option; however, there is currently no definitive treatment, such as regeneration, available for these conditions.

Stem cell therapy

Potential effects of stem cell therapy

Mesenchymal stem cells (MSCs) can be isolated from adult tissues, cultured, and possess the ability to differentiate into various cell types and exert immunomodulatory functions therefore, they have garnered attention for clinical use (Heo et al., 2016). While it remains unclear whether all transplanted MSCs differentiate into the intended cell type, they have been observed to migrate to damaged brain areas. Approximately 50% of engrafted MSCs around brain infarction areas expressed a neuronal marker 4 weeks after transplantation (Shichinohe et al., 2007). The immunomodulatory effects of MSCs are mainly attributed to interactions with immune cells through cell-to-cell contact and paracrine activity. MSCs have been shown to activate intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, which play important roles in T cell activation and leukocyte recruitment at inflammation sites (Ren et al., 2010). Gene profiling of human bone marrow-derived MSCs (BM-MSCs) has revealed high expression of galectin-1 in MSCs, and knockdown of galectin-1 significantly diminishes their immunomodulatory properties (Gieseke et al., 2010). Additionally, human BM-MSCs express high levels of Toll-like receptors (TLRs) 3 and 4, which are responsible for nuclear factor-κB (NF-κB) activity and cytokine production (Liotta et al., 2008). They have been shown to restore an efficient T-cell response to infection (Liotta et al., 2008). MSCs may also interact with B cells and macrophages. Adipose tissue-derived MSCs (AD-MSCs) have been shown to inhibit caspase 3-mediated apoptosis of B cells via vascular endothelial growth factor and block the cell cycle of B cells via activation of the p38 mitogen-activated protein kinase (MAPK) pathway (Song et al., 2020). Paracrine factors are encapsulated in extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, by size or origin (Lai et al., 2014; Song et al., 2020). EVs, as a secretome from MSCs,

contain DNA, microRNA (miR), and proteins such as cytokines, growth factors, and chemokines (Song et al., 2020). These include TGF-β1, tumor necrosis factor (TNF)-α, prostaglandin E2, interferon-γ, hepatocyte growth factor, fibroblast growth factor, indoleamine-pyrrole 2,3-dioxygenase, and nitric oxide (NO), which may exert anti-inflammatory effects (Song et al., 2020; Nakano and Fujimiya, 2021). Exosomes derived from MSCs can affect macrophage polarization (Song et al., 2020) and suppress microglia activation in the traumatic brain injury animal model (Chen et al., 2020b). Given these properties, MSCs have been considered for clinical applications in various diseases, including brain tumors, neurodegenerative diseases, and stroke (Nakano and Fujimiya, 2021).

Available stem cells

Bone marrow is the first reported and a major source of MSCs used for research. However, obtaining cells from bone marrow requires an invasive procedure, and the self-renewal ability of MSCs can decline with age (Heo et al., 2016). Recently, alternative sources of MSCs such as adipose tissue (AD-MSCs), peripheral blood, fetal tissues, dental pulp (DP-MSCs), umbilical cord (UC-MSCs), and placenta (Song et al., 2020) have been suggested for experimental and clinical use due to fewer ethical concerns and easier access to sufficient amounts (Heo et al., 2016). MSCs from different sources may exhibit varying capacities, thereby affecting their efficacy (Heo et al., 2016; Song et al., 2020). Autologous MSCs are easy to obtain and are not rejected by the immune system after transplantation. However, their establishment requires several weeks, during which they may manifest genes associated with the disease (Li et al., 2021). In contrast, allogeneic or xenogeneic MSCs offer immediate availability and cell selection advantages. However, immune rejection and quick clearance may occur after transplantation (Li et al., 2021). Pluripotent stem cells, comprising both embryonic stem cells and induced pluripotent stem cells, have been explored for potential clinical applications. Nevertheless, their widespread availability is limited by concerns regarding tumorigenicity, immunogenicity, and heterogeneity (Yamanaka, 2020). Thus, few clinical studies have been planned or completed for brain diseases. Apart from SAH, many clinical or preclinical studies using MSCs have been conducted in traumatic brain injury (Alizada et al., 2021), intracerebral hemorrhage (Huang et al., 2020), and ischemic stroke (Kawabori et al., 2020). While many preclinical studies have shown that MSC transplantation has beneficial effects for these pathologies, its efficacy in clinical settings has not been proven yet. Nevertheless, investigating the use of stem cells in SAH pathology is appealing because there is no fundamental effective treatment for brain injury caused by aneurysm rupture. This review explores the potential use of stem cells in addressing SAH pathology, examining their effectiveness as a therapeutic approach in both preclinical and clinical research concerning SAH.

Search Strategy

On January 19, 2024, a PubMed search was conducted using the search keywords (((((stem cell) OR (mesenchymal stem cell)) OR (oligodendrocyte progenitor cell)) OR (neurogenesis)) AND (subarachnoid hemorrhage)) NOT (review[Publication Type]). From this search, a total of 107 articles were retrieved and evaluated for inclusion. Among these, 70 articles were excluded as they were review articles, study protocols, or unrelated to neurogenesis or stem cell therapy. Thirty-seven original articles were included in this narrative review, categorized into neurogenesis (11 articles, including observational studies on neurogenesis in SAH pathology, shown in **Additional Table 1**), remyelination (3 articles, including observational studies on remyelination in SAH pathology, shown in **Additional Table 2**), experimental studies (22 articles, including interventions in experimental SAH models, using stem cells for treatment, shown in **Additional Table 3**), and one clinical study.

Current Evidence of Stem Cell Therapy for Subarachnoid Hemorrhage

Neurogenesis

In the adult human brain, neural stem cells (NSCs) are known to exist in two main areas: the sub-granular zone (SGZ) of the dentate gyrus of the hippocampus and the sub-ventricular zone (SVZ) of the lateral ventricles (Sgubin et al., 2007). However, it is not fully understood if this also occurs in pathological conditions like stroke (Sgubin et al., 2007). In a SAH model of adult mice, BrdU-positive cells, a marker of cell proliferation, were found to decrease in the SVZ and SGZ compared to normal controls on day 3 after SAH induction but recovered to normal levels by day 7 (Mino et al., 2003). By day 30 after SAH induction, the majority of BrdU-positive cells that migrated into the SGZ had become neurons (Mino et al., 2003). In brain specimens obtained from SAH patients during surgery, neural proliferation markers such as sex-determining region Y-box (SOX)2 and Musashi2, intermediate filament proteins like vimentin and nestin, and general cell proliferation markers such as Ki67 and proliferating cell nuclear antigen were found to be expressed, whereas they were not detected in control specimens (Sgubin et al., 2007). CSF is believed to contain proliferation-promoting factors because the SVZ and SGZ, where neurogenesis could occur, are adjacent to the lateral ventricles, allowing such factors to appear in CSF (Chen et al., 2018). Enhanced cell proliferation, differentiation, and migratory capacities were observed in the SVZ and the striatum on days 5 and 7 after SAH induction in rats. Additionally, when CSF samples taken from SAH rats on days 5 and 7 were added to NSCs of fetal rats, a similar enhancement of cell abilities was observed (Lee et al., 2016). Yue et al. (2023) reported increased expressions of NOD-like receptor thermal protein domain associated protein (NLRP) 3 and Caspase1 p20 in NSC in the hippocampus after SAH induction in mice by endovascular perforation. An *in vitro* study using NSC culture revealed that hemoglobin stimulation induced pyroptosis, and the reactive oxygen species inhibitor N-acetyl-L-cysteine inhibited pyroptosis (Yue et al., 2023).

CSF samples collected from patients with SAH on day 5 after the rupture of an aneurysm significantly increased the expression of the proliferation marker Ki67 in NSCs isolated from rat embryos (Chen et al., 2018). The expression levels of cluster of differentiation (CD)133 (prominin-1), an antigenic marker of stem cells that possibly regulate the architecture

and dynamics of cellular protrusions, were studied in patients with aneurysmal SAH and intracerebral hemorrhage (Bobinger et al., 2020). The levels of prominin-1 in the CSF obtained from these patients were significantly increased compared with healthy controls and declined during the hospital stay (Bobinger et al., 2020). The authors discussed that CD133-positive membrane particles may derive from myelin sheaths (Corbeil et al., 2009) and ependymal cells and astrocytes residing in SVZ (Bachor et al., 2017), and pathological processes such as inflammation may destroy and release them into the CSF (Bobinger et al., 2020).

In a cerebral ischemia model, low doses of 17-N-Allylamino-17-demethoxygeldanamycin (17-AAG), a potent inhibitor of heat shock protein 90, have shown potential in safeguarding neural progenitor cells against apoptosis and necrosis (Bradley et al., 2014). Notably, while this drug was primarily developed to trigger apoptosis in cancer cells, its neuroprotective effects have been observed (Bradley et al., 2014). At reduced concentrations, 17-AAG was administered to the SAH model mice, leading to an increased quantity of BrdU and doublecortin-positive cells in the hippocampus, as well as elevated levels of brain-derived neurotrophic factor (BDNF) compared to the control group (Zuo et al., 2018). Zuo et al. (2019) conducted a time-course observational study of neural progenitor cells in the hippocampus of SAH endovascular perforation model rats. They observed a decrease in BrdU-positive cells until 3 days post-SAH induction, followed by recovery within 14 days. A similar trend was observed in doublecortin-positive cells (Zuo et al., 2019). Eight weeks after SAH induction, most of the BrdU-positive cells were co-immunostained with NeuN, suggesting that progenitor cells tend to differentiate into neurons rather than astrocytes after SAH induction. In an oxyhemoglobin (OxyHb) added *in vitro* SAH mimic model, CD24 expression in hippocampal astrocytes increased after OxyHb addition. CD24 knockdown could suppress the activation of the astrocytes related to neuron growth in the hippocampus (Chen et al., 2022). Qiu et al. (2024) explored the connection between neurogenesis and ketone bodies, which are capable of crossing the BBB and acting as an alternative energy supply for brain metabolism, as well as its association with cognitive function in neurodegenerative disorders. 3-Oxoacid-CoA-transferase 1 (OXCT1), a mitochondrial enzyme that catalyzes ketone body metabolism, was shown to decrease in an SAH single blood injection model. Overexpression of OXCT1 promoted neurogenesis in the hippocampus and improved cognitive function via Akt/glycogen synthase kinase (GSK)-3β/β-catenin signaling.

In recent years, some transcription factors have been considered important in cell reprogramming. NeuroD1, one of the transcription factors that can convert astrocytes into neurons, promoted neurogenesis in the hippocampus and SGZ when injected into the hippocampus using an adeno-associated virus delivery system in the late phase after SAH induction in rats, with improved cognitive function (Chen et al., 2023).

To evaluate neurogenesis in patients with aneurysmal SAH, one study protocol has suggested collecting CSF samples from the inserted ventricular catheter in the acute phase and serially collecting blood samples until the chronic phase (Nogueira et al., 2014). The biomarkers suggested in the protocol include BDNF, epithelial growth factor, erythropoietin, fibroblast growth factor-2, granulocyte colony-stimulating factor, monocyte chemoattractant protein-1, stromal cell-derived factor-1, and vascular endothelial growth factor as molecular markers. Additionally, CD34 (endothelial progenitor cells), CD133 (endothelial and neural progenitor cells), and CD68 (microglia/monocytes) are suggested as cellular markers for the evaluation of neurogenesis in SAH patients (Nogueira et al., 2014).

Remyelination

White matter injury (WMI), a common type of brain injury in preterm neonates, can be caused by impaired oligodendrocyte maturation and myelination, resulting in decreased brain function (van Tilborg et al., 2016). Recently, similar to perinatal WMI, inflammation, and oxidative stress in the adult brain have been reported to cause demyelination. In multiple sclerosis patients, oligodendrocyte progenitor cells (OPCs) have been shown to be newly generated and migrate towards demyelinating lesions but not fully matured into developed oligodendrocytes (van Tilborg et al., 2016). Although the exact mechanisms of WMI in SAH remain unclear, SAH model mice exhibited OPCs in regions such as the SVZ, corpus callosum, and white matter, with the highest number observed between days 3 to 7 following SAH induction (Li et al., 2018). Nexilin may be a key molecule regulating remyelination (Li et al., 2018). Lipocalin-2, which has a negative effect on remyelination in multiple sclerosis patients, when knocked down, decreased the WMI area and promoted OPC differentiation in SAH mice (Li et al., 2022). Wang et al. (2003) reported that electroacupuncture, a Chinese traditional medicine, promoted remyelination in the white matter of SAH endovascular perforation model rats by decreasing Id2, which negatively affects OPC differentiation, and increasing SOX10, a transcription factor of myelination. Understanding WMI and targeting OPCs as part of stem cell therapy may be a novel approach for brain injury in SAH.

Experimental study

A total of 22 articles were included in the experimental studies, where stem cell therapy was performed on preclinical SAH models (20 articles) and experimental intracranial aneurysm models (2 articles). The most commonly used cell types were allogeneic or xenogeneic BM-MSCs and their EVs (15 articles), followed by xenogeneic (human) UC-MSCs and their EVs (3 articles). Others used included allogeneic DP-MSCs (2 articles), allogeneic AD-MSCs (1 article), and allogeneic bone marrow-derived endothelial progenitor cells (BM-EPCs) (1 article). This review includes 10 studies that used stem cells as a treatment, 10 studies that used EVs such as exosomes extracted from stem cells, and 2 studies that used conditioned medium derived from DP-MSCs. Mostly for *in vivo* SAH models, intravenous (IV) administration was performed following SAH induction.

Khalili et al. (2012) reported that IV transplantation of allogeneic BM-MSCs to SAH model rats (using a single blood

injection model) increased the expression of neural and vascular endothelial cells, reduced neural apoptosis, and improved functional recovery 14 days after SAH induction. The same group also demonstrated that in the same SAH model, IV transplantation of allogeneic BM-MSCs improved the structural integrity of cerebral tissue observed by transmission electron microscopy (Khalili et al., 2014). Additionally, IV allogeneic BM-MSCs were utilized in SAH endovascular perforation model rats, leading to a significant decrease in brain edema and suppression of microglial activation by downregulating inflammatory cytokines (Liu et al., 2019). This intervention inhibited the Notch1-related pathway, and the knockdown of the Botch gene in BM-MSCs abolished the neuroprotective effects (Liu et al., 2019).

TNF-induced protein 6 (TSG-6), potentially associated with the paracrine function of BM-MSCs and possessing anti-inflammatory properties, exhibited elevated levels in BM-MSCs following TNF-α stimulation (Wan et al., 2021). TNF-α-stimulated BM-MSCs upregulated BBB constituent proteins, reduced apoptosis, and NO production, and suppressed the expression of MAPKs and phosphorylated p65 (Wan et al., 2021). These neuroprotective effects were reversed upon *TSG-6* gene knockdown in BM-MSCs activated by lipopolysaccharide-induced astrocytes (Wan et al., 2021). Additionally, IV administration of xenogeneic (human-derived) BM-MSCs in rats with SAH endovascular perforation decreased BBB permeability and inflammatory cytokines through increased TSG-6 expression (Wan et al., 2021). Jung et al. (2023) reported that IV human BM-MSCs decreased the mRNA expression of high-mobility-group box protein 1 (HMGB1), the receptor for advanced glycation end products, TLR4, and myeloid differentiation 88, as well as protein levels of HMGB1 and NF-κB. This intervention improved cognitive function and decreased cerebral edema in SAH endovascular perforation model rats.

While IV stem cell administration is common in clinical practice, intranasal administration can also be chosen as an alternative route because it may lead to a high accumulation of cells in peripheral tissues. Additionally, a higher number of stem cells might be required to achieve satisfactory results (Nijboer et al., 2018). Intranasal administration of allogeneic BM-MSCs to rats, 6 days after SAH induction by endovascular perforation improved sensorimotor, mechanosensory, and depression-like behavior in relation to tyrosine hydroxylase (Nijboer et al., 2018). Furthermore, it reduced the loss of gray and white matter volume and decreased the activation of astrocytes, microglia, and macrophages (Nijboer et al., 2018). BM-MSCs treatment has also been utilized for *in vitro* studies. NO production was reduced in OxyHb-treated microglia cells mimicking SAH when co-cultured with BM-MSCs (Zhang et al., 2020). Moreover, it modulated microglial polarization and reduced the expression of inflammatory genes such as TNF-α and interleukin-1β (Zhang et al., 2020).

Several groups selected EVs extracted from BM-MSCs to evaluate their beneficial effects on SAH models. IV administration of EVs improved neurobehavior and suppressed neuronal apoptosis in rats in the SAH endovascular perforation model. An *in vitro* study using OxyHb-treated primary rat neurons has shown that miR-21 in EVs is considered to be an important factor in the neuroprotective effects (Gao et al., 2020). Xiong et al. (2020) focused on miR-129-5p found in EVs derived from allogeneic BM-MSCs, identifying it as a crucial element contributing to neuroprotective effects. IV administration of exosomes isolated from allogeneic BM-MSCs led to improved neurological outcomes and reduced brain edema in rats with SAH induced by endovascular perforation. However, when miR-129-5p was inhibited, the neuroprotective effects were reversed, and the exosome treatment lowered the expression of HMGB1, TLR4, and p53, all of which are known to be upregulated following SAH (Xiong et al., 2020). Han et al. (2021) investigated whether EVs derived from allogeneic BM-MSCs relate to microglia polarization. IV administration of EVs derived from allogeneic BM-MSCs improved neurological scores and brain edema, decreased the number of microglia in the parietal cortex and hippocampus, and reduced expressions of interleukin-1β, inducible NO synthase, CD11b, CD16, phosphorylated nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha, and NF-κB. Moreover, they increased expressions of phosphorylated adenosine 5′-monophosphate-activated protein kinase, TGF-β, Arg-1, and CD206. Taken together, these findings suggest that the polarization of microglia might be modulated by EVs (Han et al., 2021). Activin-like kinase 5, which is a potential target of miR-140-5p, when silenced in SAH endovascular perforation model mice, improved neurological outcome, reduced neuronal apoptosis, and inflammation (Qian et al., 2022). EVs derived from allogeneic BM-MSCs with miR-140-5p mimic reduced neuronal apoptosis and alleviated inflammation by suppressing microglia activation (Qian et al., 2022). Rabies virus glycoprotein, engineered on the exosomal surface to cross the BBB and transport miR specifically into the brain, modified exosomes derived from allogeneic BM-MSCs mixed with miR-193b-3p mimics, improving neurological function, brain edema, and BBB disruption, and suppressing apoptotic and inflammatory molecules by inhibiting the histone deacetylase 3/NF-κB signal pathway in SAH model mice (Lai et al., 2020). Cheng et al. (2022) reported that IV administration of EVs from allogeneic BM-MSCs improved brain edema in SAH endovascular perforation model rats. Gene expression profiling in SAH patients and an *in vitro* SAH mimic study using brain microvascular endothelial cells confirmed that Krüppel-like factor 3 antisense 1, downregulated in the samples from aneurysmal SAH patients and possibly existing in exosomes derived from MSCs, is beneficial via miR-83-5p contained in EVs from BM-MSCs. Zhang et al. (2023) reported that intracerebroventricular administration of EVs derived from allogeneic BM-MSCs improved neurological status by reducing markers of inflammation, endoplasmic reticulum stress, and oxidative stress, possibly through the effect of miR-18a-5p in a SAH endovascular perforation model in rats. Additionally, an *in vitro* study using hypoxia/reoxygenation-induced brain cortical neurons confirmed these effects.

Intracerebroventricular administration of human UC-MSCs one day before surgery reduced levels of inflammatory cytokines and improved neurobehavior and chronic hydrocephalus in a SAH single blood injection model in rats (Chen et al.,

2020a). Knockdown of TGF-β1 in UC-MSCs enhanced these beneficial effects (Chen et al., 2020a). Exosomes extracted from human UC-MSCs improved neurological score and brain edema, suppressed apoptosis, and inhibiting miR-206 in the exosomes enhanced the neuroprotective effects via BDNF activation in SAH rats (double blood injection model) (Zhao et al., 2019). Another study reported that exosomes extracted from human UC-MSCs decreased apoptosis and the expression of inflammatory mediators in OxyHb-induced SAH mimic neurons (Liu et al., 2021b). The inhibition of miR-26b-5p reversed these beneficial effects, suggesting that miR-26b-5p may be an important factor in the exosomes (Liu et al., 2021b). Overexpression of methionine adenosyltransferase 2A, which is essential for cell growth and differentiation and potentially the target of miR-26b-5p and related to inflammatory reaction, also abolished the anti-apoptotic and anti-inflammatory effects of exosomes derived from human UC-MSCs (Liu et al., 2021b). IV administration of exosomes derived from human UC-MSCs improved neurobehavioral scores and decreased brain water content, as well as the expression levels of p38 MAPK and signal transducer and activator of transcription 3. These effects were reversed by miR-26b-5p inhibitor in a SAH double blood injection model in rats (Liu et al., 2021b).

Chen et al. (2019) utilized allogeneic DP-MSCs derived conditioned media via intrathecal administration. This approach alleviated vasoconstriction and reduced the presence of microglia, although the effects were partially counteracted by the administration of an insulin-like growth factor-1 antibody. Furthermore, the treatment demonstrated an enhancement in neurological function, as assessed through the rotarod test. Yang et al. (2022) also employed intrathecal allogeneic DP-MSCs derived conditioned media, which decreased brain edema, improved microcirculation impairment, and neurological score in SAH single blood injection model rats by promoting M2 microglia polarization and reducing astrocytic swelling. The authors concluded that insulin-like growth factor-1 in DP-MSCs derived conditioned media may play an important role in its beneficial effects.

One report used exosomes extracted from allogeneic AD-MSCs, which decreased apoptosis in OxyHb-induced SAH mimic primary neurons and SAH endovascular perforation model rats via miR-140-5p, contained in AD-MSCs-derived exosomes (Wang et al., 2022).

Two reports utilized stem cells for an experimental aneurysm model to investigate whether stem cell administration prevents aneurysms from rupture. IV administration of allogeneic BM-EPCs to stromal cell-derived factor-1α-coated coil-treated experimental aneurysm model rats promoted fibrosis and occlusion of aneurysms (Gao et al., 2014). In another study, IV administration of human BM-MSCs reduced the aneurysm rupture rate *in vivo* (Kuwabara et al., 2017). Additionally, an *in vitro* study showed that the release of proinflammatory cytokines from mast cells decreased via cyclooxygenase-2 when co-cultured with BM-MSCs (Kuwabara et al., 2017).

Clinical study

Clinical studies related to SAH are limited. Only one case report has been published in which allogeneic BM-MSCs were used for an 80-year-old male patient who suffered from high-grade aneurysmal SAH (Brunet et al., 2019). On the third day after onset, the patient received a single IV injection of 1×10^8 human allogeneic BM-MSCs in 80 mL at a rate of 2 mL/min over 45 minutes with no allergic or adverse events (Brunet et al., 2019). The authors concluded that the therapy was deemed effective because the modified Rankin Scale score at 6 months after onset was 3, while his initial symptoms were severe (Brunet et al., 2019). Based on a search on ClinicalTrials.gov with the keywords "subarachnoid hemorrhage" and "stem cell" (performed on January 19, 2024), currently there are no trials completed or recruiting patients for stem cell therapy, while a number of clinical trials were completed or recruiting patients for traumatic brain injury, intracerebral hemorrhage, and ischemic stroke.

Perspective and Conclusion

A small number of studies have been published on SAH pathology and stem cells, so it is not conclusive whether stem cell therapy is beneficial. Moreover, several issues need to be addressed before clinical usage. Firstly, the types of stem cells should be discussed. BM-MSCs have been the most common in preclinical studies, but the harvesting method is invasive, and age-related declines in stem cell capacity may be observed. Therefore, alternative sources such as adipose tissue, placenta, and umbilical cord have been increasingly investigated (Heo et al., 2016). Furthermore, the choice between using cells themselves or EVs for administration should also be discussed. Secondly, the appropriate administration route is not confirmed. The most commonly performed route is IV, but systemic usage might require a larger number of stem cells, leading to accumulation in peripheral tissues. Alternatively, intrathecal or intraparenchymal administration can be selected for specific purposes, although it can be invasive. Alternatively, intranasal administration can be selected because it potentially bypasses the BBB in a non-invasive manner (Donega et al., 2013; Nijboer et al., 2018). Adjusting the amount of stem cells may also be necessary based on the chosen administration route. Thirdly, the timing of transplantation is crucial. Transplantation during the acute phase after SAH requires allogeneic stem cells, and careful observation is necessary for potential adverse effects. Autologous stem cells can be harvested after onset and may be used in the chronic phase after SAH to promote tissue repair and regeneration with a smaller risk of complications.

While the precise mechanisms underlying the beneficial effects of stem cells remain unclear, previous studies have shown that certain properties, such as their anti-inflammatory effects, may be effective for SAH. Intrinsic ligands containing damage-associated molecular patterns, such as heme, fibrinogen, and intracellular components released at tissue damage sites, initially activate pattern recognition receptors, including TLR4 (Kanamaru et al., 2019). This activation marks the onset of inflammatory cascades triggered by aneurysm rupture and SAH (Kanamaru et al., 2019). Most cells in the brain, vascular

wall, inflammatory cells, and platelets express TLR4, which has been reported to induce maximal inflammatory responses among all TLR family members (Kanamaru et al., 2019; Suzuki et al., 2020). Upon activation, TLR4 triggers NF-κB and MAPK pathways, initiating inflammatory responses characterized by the release of interleukins, matrix metalloproteinase-9, reactive oxygen species, and the upregulation of matricellular proteins (MCPs), including tenascin-C (TNC) and periostin (Kanamaru et al., 2019). Our group has demonstrated the association between TLR4 activation and the pathologies of neuroinflammation, BBB disruption, neuronal apoptosis, and cerebral vasospasm in an SAH animal model (Nishikawa et al., 2018; Kanamaru et al., 2019). We also investigated the role of certain inflammatory molecules known as MCPs, which are inducible and secreted non-structural extracellular matrix proteins (Kawakita et al., 2019). The extracellular matrix, comprising approximately 20% of the brain and consisting of glycoproteins and proteoglycans, potentially plays important roles during development and regeneration (Faissner et al., 2017; Cope and Gould, 2019). Since some MCPs are known to upregulate and activate TLR4, their activation can initiate inflammatory cascades (Kawakita et al., 2019), which may be influenced by stem cell therapies. TNC, as one of the MCPs, can activate TLR4 and upregulate TNC itself via the NF-κB and MAPK pathways, creating a positive feedback loop that further facilitates inflammatory reactions after SAH (Suzuki et al., 2020). Previous studies have suggested that TNC plays an important role in the proliferation and differentiation of NSCs and glial progenitor cells (Faissner et al., 2017). Therefore, TNC expression might affect the outcome of stem cell therapy.

Periostin, one of the MCPs that can increase in injured brain tissues, demonstrates promise due to its interaction with TNC and activation of TLR4-related pathways alongside TNC in SAH model mice (Okada et al., 2019). Additionally, galectin-3, another MCP, may be involved in BBB disruption in SAH model mice, and its inhibition has been found to improve brain edema (Nishikawa et al., 2018). Galectin-3 has been implicated in cancer pathologies. For instance, when human UC-MSCs derived exosomes carrying miR-128-3p were used for pancreatic cancer cells, the proliferation, invasion, and migration of the cancer cells *in vitro* were suppressed by inhibiting galectin-3 (Xie et al., 2022). Based on these findings, MCPs can be targets of stem cell therapy.

In conclusion, previous studies have shown that stem cell therapy holds promise as a treatment for SAH patients. In a clinical setting, the administration protocol, including timing, frequency, route of administration, and evaluation methods such as detailed neurological status and neuroregenerative biomarkers, should be carefully considered. To clarify the mechanism of potential stem cell therapy, pre-clinical investigations are also necessary to determine how long *in vivo* application of stem cells survives after administration and which origin of stem cells or their EVs should be used before clinical application. Despite these limitations for clinical application, stem cell therapy remains an attractive treatment option for currently incurable brain injuries after SAH.

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Additional files:

Additional Table 1: *Articles of neurogenesis on SAH.*

Additional Table 2: *Articles of remyelination on SAH.*

Additional Table 3: *Articles of experimental studies that use stem cells as a treatment on in vivo or in vitro models of aneurysm or SAH.*

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