Research Article

Inhibition of Cerebral Ischemia/Reperfusion Injury by MSCs-Derived Small Extracellular Vesicles in Rodent Models: A Systematic Review and Meta-Analysis

Lei Zhang, Chaoying Pei, Dan Hou, Guoshuai Yang, and Dan Yu

Department of Neurology, Affiliated Haikou Hospital of Xiangya School of Medicine, Central South University, Haikou 570208, China

Correspondence should be addressed to Guoshuai Yang; youngester4213@sina.com and Dan Yu; yudanyuyue@163.com

Received 13 May 2022; Accepted 17 September 2022; Published 6 October 2022

Academic Editor: Gabriela Delevati Colpo

Copyright © 2022 Lei Zhang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Small extracellular vesicles (sEVs) secreted by mesenchymal stem cells (MSCs) have shown great therapeutic potential in cerebral ischemia-reperfusion injury (CIRI). In this study, we first performed a systematic review to evaluate the efficacy of MSCs-derived sEV for experimental cerebral ischemia/reperfusion injury. 24 studies were identified by searching 8 databases from January 2012 to August 2022. The methodological quality was assessed by using the SYRCLE’s risk of bias tool for animal studies. All the data were analyzed using RevMan 5.3 software. As a result, the score of study quality ranged from 3 to 9 in a total of ten points. Meta-analyses showed that MSCs-derived sEVs could effectively alleviate neurological impairment scores, reduced the volume of cerebral infarction and brain water content, and attenuated neuronal apoptosis. Additionally, the possible mechanisms of MSCs-derived sEVs for attenuating neuronal apoptosis were inhibiting microglia-mediated neuroinflammation. Thus, MSCs-derived sEVs might be regarded as a novel insight for cerebral ischemic stroke. However, further mechanistic studies, therapeutic safety, and clinical trials are required. Systematic review registration. PROSPERO CRD42022312227.

1. Introduction

Stroke has the characteristics of high economic burden, high incidence, high recurrence rate, high mortality rate, and high disability rate, among which the incidence of ischemic stroke (IS) is the highest [1]. Treatment of acute ischemic stroke (AIS) is based on the timely restoration of blood flow to the ischemic brain tissue by intravenous thrombolysis (IVT) and/or mechanical thrombectomy (MT) [2]. However, recanalization may aggravate the neurological deficit after cerebral ischemia, that is, cerebral ischemia-reperfusion injury (CIRI).

CIRI refers to the phenomenon that ischemic injury of the brain leads to the injury of brain cells, which is further aggravated after the recovery of blood reperfusion [3]. The specific mechanism may be related to oxygen free radicals through lipid peroxidation, protein degeneration, mitochondrial apoptosis, and activation of death receptors during reperfusion [3]. This kind of injury can further lead to aggravation of brain injury and neurological dysfunction, and even nerve cell death [4]. At present, CIRI has attracted increasing attention. However, neuroprotective drugs used to treat the neuronal injury caused by CIRI have limited therapeutic effects. It is of great significance to develop a more effective new approach for the treatment of CIRI.

Mesenchymal stem cells (MSCs) possess the characteristics of immunoregulation, multidirectional differentiation potential, easy access, rapid proliferation in vitro, low activity loss after cryopreservation, low immunogenicity, and nontoxic side effects [5]. Several previous studies have demonstrated tremendous potential of MSCs in treating CIRI [6], myocardial ischemia-reperfusion injury (IRI) [7], hepatic IRI [8], intestinal IRI [9], renal IRI [10], lung IRI [11], retinal IRI [12], and spinal cord IRI [13] exhibiting specific mechanisms of action, such as angiogenesis, antiapoptosis, anti-inflammation, and tissue regeneration. In recent years, the literature supports that the paracrine mechanism
of MSCs is mediated at least in part by extracellular vesicles (EVs) [14].

Extracellular vesicles (EVs) are membrane vesicles that are released into the surrounding extracellular environment and can be divided into the subgroups of microvesicles and exosomes [15, 16]. Exosomes are vesicles released by a cell that is between 30 and 100 nm in diameter, which is composed of a multiprotein complex, containing receptors, enzymes, transcription factors, extracellular matrix (ECM) proteins, nucleic acids (mtDNA, ssDNA, dsDNA, mRNA, and miRNA), and also lipids [17–19]. Previous studies have reported that exosomes showed similar or equivalent therapeutic function to MSCs to reduce injury caused by ischemia/reperfusion in a variety of tissues and organs, including the spinal cord [20], kidney [21], liver [22], heart [23], lung [24], brain [25–49], and intestine [50]. Treatment with exosomes overcomes the limitations associated with cell-based therapies and offers several advantages such as easy entry into the ischemic brain after their administration owing to their lipophilicity, less or no immunogenicity and tumorigenicity, and less incidence of occlusion in the microvasculature [27]. Due to difficulties existing in the isolation of a pure population of exosomes through the method used in present studies, we will use the term “small extracellular vesicles” (sEVs) to refer to EVs less than 200 nm in diameter, according to the updated guidelines of the International Society for Extracellular Vesicles of 2018 (MISEV2018) [51].

In this study, 24 published literatures [26–49] were systematically reviewed and meta-analyzed to evaluate the safety and efficacy of exocrine derived from mesenchymal stem cells in the treatment of CIRI. Thus, it can provide a reference basis for the clinical use of exocrine derived from mesenchymal stem cells in the future treatment of CIRI and put it into clinical applications in a timely manner.

2. Methods

2.1. Search Strategies. Relevant papers published between January 2012 and August 2022 were screened in PubMed, Web of Science, Embase, Cochrane Library, CNKI (China National Knowledge Infrastructure), VIP Database for Chinese Technical Periodicals, Wanfang Database, and Chinese Biomedical Literature Database. The following keywords were used for literature retrieval: “exosomes”, “extracellular vesicles”, “EVs”, “Reperfusion Injury”, “MSCs”, and “Mesenchymal Stem Cells.” All searches used combinations of keywords and free words, while appropriate adjustments were made according to the corresponding database. Relevant systematic reviews and references cited in the searched articles were also filtered to avoid leaving out any potentially usable studies. Besides, other related articles were also available by examining the reference list by hand. There is no restriction on publication language or publication status. Take PubMed as an example, the specific retrieval strategies are shown in the Supplementary 2 File.

2.2. Inclusion and Exclusion Criteria. Studies meeting the following criteria at the same time were included in this paper: (1) animal model: rats or mice of any age or gender exposed to cerebral ischemia-reperfusion injury; (2) intervention: sEVs derived from mesenchymal stem cells without any restriction on the source of cells, the administration dose, and the site of transplantation; (3) comparison: saline, phosphate buffer saline (PBS), or no treatment; (4) outcome measure: cerebral infarct volume, apoptosis rate, neurologic impairment scores, brain water content, Caspase-3, tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6). Articles meeting any of the following criteria were excluded: (1) duplicate literature; (2) reviews, conference abstracts, editorials, letters to editors, case reports, and other meta-analyses; (3) studies in humans, or in vitro studies; (4) other sEVs than derived from mesenchymal stem cells; (5) incorrect or incomplete literature data that could not be included in the statistical analysis; (6) No relevant outcomes reported.

2.3. Quality Assessment. The quality assessment of the studies included in the present research was independently performed by two researchers using SYRCLE’s risk of bias tool for animal studies [52] recommending ten items of evaluation, evaluation results with “Y”, “N” and “U” represent, respectively, low risk of bias, bias risk, and uncertain risk of bias. The disagreements between the 2 investigators were settled by means of discussion until an agreement was reached with the third investigator.

2.4. Data Extraction. Two independent authors extracted the following details from the included studies and made a data extraction sheet: (1) the name of the first author; (2) year of publication; (3) country; (4) animal species, sex, and weight; (5) kind of anesthetic; (6) the source of MSCs; (7) MSCs isolation method; (8) MSCs characterization method; (9) MSCs positive marker; (10) EVs isolation method; (11) EVs characterization method; (12) the diameter of EVs; (13) EVs positive marker; (14) model of cerebral I/R; (15) the information of treatment group, including therapeutic drug dosage, method of administration, duration of treatment, and the same information of control group; (16) time point of extracting brain tissue; (17) mean value and standard deviation of outcomes. When the response was not received, the numerical values were measured from the graphs by GetData Graph Digitizer 2.26 software.

2.5. Statistical Analysis. The pooled analyses were carried out with RevMan 5.3 software. I^2 statistics is calculated and reported to assess the degree of heterogeneity. A fixed-effects model (I^2<50%) or a random-effects model (I^2>50%) was used depending on the value of I^2. Funnel plots were used to visually estimate publication bias. We calculated the standard mean difference (SMD) with 95% confidence intervals (CIs).
3. Results

3.1. Study Selection. A total of 1116 articles were retrieved through the pertinent literature retrieval from the database, of which 421 were reduplicated or before January 2012 articles. After screening titles and abstracts, 636 were excluded because they were (1) reviews, conference abstracts, editorials, letters to the editor, case reports, and other meta-analyses and (2) records associated with other tissues. We then studied the remaining 59 full-text articles. Among them, 35 articles were excluded for at least one of the following reasons: (1) not full text; (2) not MSCs-derived sEVs; (3) not in rats or mice vivo study; (4) not RCT; (5) no relevant outcomes reported. Finally, 24 studies [26–49] were selected (Figure 1).

3.2. Characteristics of Included Studies. A cerebral I/R model for Balb/C mice was constructed in one of these researches [32] while Sprague-Dawley (SD) rats were the subjects of 16 experiments [26–31, 34, 35, 39, 40, 42, 44, 46–49], and C57BL/6J mice were the subjects of 7 studies [33, 36–38, 41, 43, 45]. Only male animals were employed in 19 researches [26–31, 34, 35, 38–48]; other studies [32, 33, 36, 37, 49] did not report the sex of the animals. Anesthesia: 9 studies [26, 29, 30, 34, 35, 37, 38, 41, 47] used pentobarbital; 3 studies [28, 31, 48] used chloral hydrate; 1 study [36] used chloral hydrate and xylazine; 5 studies [27, 39, 40, 43, 46] used isoflurane; 1 study [32] used ketamine; 1 study [44] used ether; 1 study [45] used isoflurane oxygen/nitrous oxide mixture; 1 study [49] used uratan; and 2 studies [33, 42] not clearly named the anesthetics used. The middle cerebral artery occlusion (MCAO) model is used as I/R model in all studies. MSCs-derived seEVs were injected into the experimental group, the control group was injected with phosphate-buffered saline (PBS) or saline, 18 studies [27, 28, 30–38, 40, 43–48] via tail vein, 5 studies [26, 29, 41, 42, 49] via the lateral cerebral ventricle, and 1 study [39] via vein. The overall characteristics of included publications are shown in Table 1.

3.3. Isolation, Characterization, and Quantification of MSCs and EVs. MSCs were isolated via centrifugation alone (7 studies) [26, 33, 38, 39, 46, 48, 49]; by gradient centrifugate method (2 studies) [35, 43]; or by adherence method (2 studies) [30, 36]; or by centrifugation in combination with filtration methods (1 study) [40]; or by centrifugation methods (1 study) [28]. 11 studies [27, 29, 31, 32, 34, 37, 41, 42, 44, 45, 47] did not report the methods used to isolate MSCs. 16 studies (67%) characterized MSCs for MSCs positive markers [26, 28, 32–38, 41–46, 49]. MSCs were fixed and stained with Alizarin Red S for osteogenic differentiation and/or Oil Red O for adipogenic differentiation in 8 studies [28, 32, 33, 38, 41, 42, 44, 45] (Table 1).

EVs were isolated via ultracentrifugation (UC) alone (12 studies) [29, 31, 34, 35, 37, 38, 41–44, 46, 49]; by UC in combination with filtration methods and/or isolation kits (4 studies) [30, 32, 40, 47]; or by isolation kits (1 study) [45]; or by isolation kits in combination with low-speed centrifugation steps (1 study) [27]; or by sequential centrifugation (1 study) [36]; or by centrifugation (4 studies) [26, 28, 33, 48]. One study [39] did not report the methods used to isolate EVs. 23 studies [26, 28–49] (95.8%) characterized EVs using transmission electron microscopy (TEM) in combination with nanoparticle tracking analysis (NTA; 9 studies) [32–35, 38, 42–45] or dynamic light scattering analysis (DLS; 1 study) [41]. 24 studies [26–49] (100%) characterized EVs using western blot for protein markers in combination with flow cytometry (3 studies) [30, 36, 47] or bicinchoninic acid (BCA) protein (1 study) [32]. In addition, 22 studies [26, 28–38, 40–49] (91.7%) reported a range in EV size from
Table 1: Characteristics of the 13 included studies.

| ID | Study | Country | Animals | Anesthetic | The source of MSCs | MSCs characterization method | MSCs positive marker | EVs isolation method | EVs characterization method | EVs positive marker | Injury | Experimental group treatment | Control group treatment | Time point of extracting brain tissue | Route | Outcomes |
|----|-------|---------|---------|------------|-------------------|-----------------|-----------------|-------------------|-----------------|-----------------|---------|-----------------------------|-------------------|-------------------------------|------|----------|
| 1  | B. Feng [36] | China | Male mice (C57BL/6 J, 22-25 g) | Sodium pentobarbital | Bone marrow | Adherence method | Flow cytometry, alizarin red staining, Alcian blue staining | Ultra-centrifugation | TEM, NTA, western blot | 30-130 nm | CD9, CD63, TSG101 | MCA occlusion for 1 hour | 200 μg EVs | PRS | Via tail vein | Infarct volume, apoptosis |
| 2  | C. Cheng [36] | China | Male mice (C57BL/6 J, 8 weeks, 250 g) | Cholined hydrate and xylazine | Bone marrow | Centrifugation | Flow cytometry, alizarin red staining, Alcian blue staining | Ultra-centrifugation | TEM, NTA, western blot | 30-130 nm | CD9, CD63, TSG101 | MCA occlusion for 1 hour | 200 μL MSCs-EVs | Saline | Not shown | Via tail vein | Infarct volume, apoptosis, Caspase-3 |
| 3  | G. Li [32] | China | Male mice (B6C3F1, 4 weeks, 20 ± 5 g) | Ketamine | Umbilical cord | Not stated | Flow cytometry, alizarin red staining, Alcian blue staining | Ultra-centrifugation | TEM, NTA, western blot, BCA protein | 30-130 nm | CD9, CD63, CD81 | MCA occlusion | 50 μg/ml bU1CMSCs-exos | PRS | Via tail vein | Neurological deficit score, infarct size, water content, TNF-α, IL-6 |
| 4  | H. Hao [36] | China | Male mice (SD, 8 weeks, 270-300 g) | Cholined hydrate | Oil red staining, alizarin red staining | Centrifugation | Flow cytometry, alizarin red staining, Alcian blue staining | Ultra-centrifugation | TEM, western blot | 30-130 nm | CD9, CD63, TSG101 | MCA occlusion for 2 hours | 100 μg of ADMSCs-exos | PRS | Via tail vein | Neurological impairment scores, cerebral infarction volume, apoptosis |
| 5  | H. Yang [45] | China | Male mice (C57BL/6 J, 20-25 g) | Isoflurane or nitrous oxide mixture | Bone marrow | Not stated | Flow cytometry, alizarin red staining, Alcian blue staining | Isolation kits | TEM, western blot | 40-130 nm | CD9, CD63, TSG101 | MCA occlusion for 2 hours | 100 μg exosomes | PRS | Via tail vein | Neurological deficit score, infarct volume, apoptosis, TNF-α, IL-1β, IL-6 |
| 6  | H. Yu [48] | China | Rat (SD, 250-270 g) | Ketamine | Rat bone marrow | Centrifugation | Flow cytometry | Ultra-centrifugation | TEM, western blot | 40-130 nm | CD9, CD63, TSG101 | MCA occlusion for 2 hours | 100 μg exosomes | PRS | Via the lateral cerebral ventricle | Neurological deficit score, infarct volume, apoptosis, TNF-α, IL-1β, IL-6 |
| 7  | K. Chen [39] | China | Male rats (SD, 350-375 g) | Inhalational isoflurane | Mini pigs adipose tissues | Centrifugation | Flow cytometry | Not stated | Not stated | Not stated | Not stated | Not stated | Not stated | Via tail vein | Infarct volume, Caspase-3, TNF-α, IL-1β |
| 8  | K. Hou [29] | China | Male rats (SD, 6-8 weeks, 250 ± 12 g) | Isoflurane | Bone marrow | Not stated | Flow cytometry | Ultra-centrifugation | TEM, western blot | 30-200 nm | CD90, CD63, TSG101 | MCA occlusion for 3 days | 10 μg/kg/d IL-6 | Saline | Via the lateral cerebral ventricle | Neurological deficit score, apopotic level, Caspase-3 |
| 9  | K. R. Nakamoto [27] | USA | Adult male rats (SD, 240 ± 20 g) | Isoflurane | Bone marrow | Not stated | Flow cytometry | Ultra-centrifugation | TEM, western blot | 30-200 nm | CD90, CD63, TSG101 | MCA occlusion for 3 days | 10 μg/kg/d IL-6 | Saline | Via the lateral cerebral ventricle | Neurological deficit score, apopotic level, Caspase-3 |
| 10 | L. Xu [33] | China | Male mice (C57BL/6 J, 25 ± 2 g) | Isoflurane | Adipose tissues | Centrifugation | Flow cytometry, oil red O staining | Centrifugation | TEM, western blot | 30-130 nm | CD90, CD63, TSG101 | MCA occlusion for 1 hour | 100 μg of MSCs-EVs | PRS | Via tail vein | Neurological deficit score, infarct size, cytokine content, apoptosis |
| 11 | M. Han [40] | China | Male rats (SD, 7-8 weeks, 30 ± 30 g) | Isoflurane | Bone marrow | Filtration, centrifugation | Flow cytometry | Ultra-centrifugation | TEM, western blot | 50-200 nm | CD90, CD63, TSG101 | MCA occlusion for 2 hours | 100 μg MSCs-EVs | PRS | Via tail vein | Neurological deficit score, infarct size, cytokine content, apoptosis |
| ID | Study | Country | Animals | Anesthetic | The source of MSCs | MSCs isolation method | MSCs characterization method | MSCs positive marker | EVs isolation method | EVs characterization method | EVs positive marker | Injury | Experimental group treatment | Control group treatment | Time point of extracting brain tissue | Route | Outcomes |
|----|-------|---------|---------|------------|-------------------|---------------------|-----------------------|----------------------|---------------------|-----------------------|---------------------|--------|--------------------------|-------------------------|-----------------------------------|--------|----------|
| 12 | Q. Pan | China   | Male mice (C37BL/6, 6–8 weeks) | Isoflurane | Mouse bone marrow | Gradient centrifuge method | Flow cytometry | CD31, CD54 | Ultracentrifugation | TEM, NTA, western blot, CD63, TSG101 | MCA occlusion for 2 hours | 1 x 10^10 particles MSC-Exs | PBS | 48 hours after reperfusion | Via tail | Infarct volume, water content |
| 13 | W. Wang | China   | Male mice (SD, 200–250 g) | Ether | Human umbilical cord | Not stated | Flow cytometry, flow cytometry | CD29, CD34, CD45 | Ultracentrifugation | TEM, NTA, western blot | MCA occlusion for 90 min | 100 μg/kg/day * 3 days | HMC-EV | PBS | 72 hours after reperfusion | Via tail | Infarct volume, apoptosis, Caspase-3, TNF-α, IL-1β, IL-6 |
| 14 | X. Huang | China   | Male mice (SD, 200–250 g) | Sodium pentobarbital | Bone marrow from normal rats | Ultracentrifugation, flow cytometry | Immunofluorescence staining, TEM, western blot | CD29, CD45 | Ultracentrifugation | TEM, western blot | MCA occlusion for 60 min | 100 μg/kg/day * 3 days | CD44, TSG101 | PBS | 72 hours after reperfusion | Via the lateral ventricle | Infarct volume, apoptotic Caspase-3 |
| 15 | X. Li | China   | Male mice (SD, 250–270 g) | Sodium pentobarbital | Bone marrow cavity of rats | Not stated | Flow cytometry | CD31, CD44, CD105 | Ultracentrifugation | TEM, NTA, western blot, CD63, TSG101 | MCA occlusion for 2 hours | 100 μg | PBS | 72 hours after reperfusion | Via tail | mNSS score |
| 16 | X. Li | China   | Male mice (SD, 200–250 g) | Sodium pentobarbital | Bone marrow cavity of rats | Not stated | Flow cytometry | CD31, CD44, CD105 | Ultracentrifugation | TEM, NTA, western blot | MCA occlusion for 2 hours | 100 μg | PBS | 72 hours after reperfusion | Via the lateral ventricle | Neurological function score, apoptosis, TNF-α, IL-1β, IL-6 |
| 17 | X. Liu | China   | Male mice (SD, 200–250 g) | Sodium pentobarbital | Bone marrow cavity of rats | Not stated | Flow cytometry | CD31, CD44, CD105 | Ultracentrifugation | TEM, NTA, western blot | MCA occlusion for 2 hours | 130 μg of BMSCs-exosomes in 2 ml PBS | PBS | 24 hours after reperfusion | Via tail | Neurological impairment scores, brain water content, cerebral infection volume, IL-1β |
| 18 | Y. An | China   | Male mice (SD, 200–250 g) | Cholecalciferol | Human umbilical cord | Centrifugation | Not stated | Not stated | Centrifugation | TEM, western blot | MCA occlusion for 2 hours | Not shown | PBS | 14 days after reperfusion | Via tail | Neurological deficit score, infarct volume |
| 19 | Y. Ye | China   | Male mice (SD, 200–250 g) | Isoflurane | Human umbilical cord | Centrifugation | Not stated | Not stated | Centrifugation | TEM, western blot | MCA occlusion for 2 hours | 80 μg * 3 days | PBS | 48 hours after reperfusion | Via tail | Neurological deficit score, infarct volume, apoptosis |
| 20 | Y. Zhao | China   | Male mice (SD, 200–250 g) | Sodium pentobarbital | Bone marrow | Adherence method | Not stated | Not stated | Ultrafiltration, TEM, western blot | MCA occlusion for 90 min | 200 μl MSC-Exosomes | Saline | 7 days after reperfusion | Via tail | Neurological severity scores (NSS) |
| 21 | Y. Zhao | China   | Male mice (C37BL/6, 6–8 weeks) | Sodium pentobarbital | Bone marrow | Not stated | Not stated | Not stated | Ultrafiltration, TEM, western blot | MCA occlusion for 90 min | 200 μl MSC-Exosomes | Normal saline | 28 days after reperfusion | Via tail | Infarct volume, IL-1β, IL-6 |
| 22 | Z. He | China   | Male mice (C37BL/6, 6–8 weeks) | Sodium pentobarbital | Bone marrow | Not stated | Not stated | Not stated | Ultrafiltration, TEM, western blot | MCA occlusion for 1 hour | 100 mmol/l * 3 days | PBS | 72 hours after reperfusion | Via tail | Infarct volume, apoptosis |
| 23 | Z. Pan | China   | Male mice (C37BL/6, 6–8 weeks) | Cholecalciferol | Human umbilical cord | Not stated | Not stated | Not stated | Ultracentrifugation | TEM, western blot | MCA occlusion | 100 μg | PBS | 24 hours after reperfusion | Via tail | Neurological impairment scores, cerebral infarction |
| ID | Study | Country | Animals | Anesthetic | The source of MSCs | MSCs isolation method | MSCs characterization method | MSCs positive marker | EVs isolation method | EVs characterization method | Diameter of EVs | EVs positive marker | Injury | Experimental group treatment | Control group treatment | Time point of extracting brain tissue | Route | Outcomes |
|----|-------|---------|---------|------------|-------------------|----------------------|------------------------|----------------------|---------------------|------------------------|----------------|----------------|--------|----------------------------|--------------------|----------------------------------|-------|---------|
| 24 | Z. Zhang [37] | China | Mice (C57/6, 8 weeks, 20-30 g) | Sodium pentobarbital | Human umbilical cord | Not stated | Flow cytometry | CD73, CD105, CD90 | Ultracentrifugation | TEM, western blot | 30-130 nm | CD9, Alix, TSG101 | MCA occlusion for 1 hour | 50 μg exosomes | PRS | 72 hours after reperfusion | Via tail vein | Neurological function scores, infarct volume, TNF-α, IL-1β, IL-6 |
Table 2: Risk of bias of the included studies.

| ID | Study                  | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ | ⑨ | ⑩ | Score |
|----|------------------------|----|----|----|----|----|----|----|----|----|----|-------|
| 1  | B. Feng [38]           | U  | Y  | U  | U  | Y  | Y  | U  | U  | Y  | Y  | 6     |
| 2  | C. Cheng [36]          | U  | U  | U  | U  | U  | U  | Y  | Y  | Y  | Y  | 3     |
| 3  | G. Li [32]             | U  | U  | U  | Y  | U  | U  | Y  | Y  | Y  | Y  | 3     |
| 4  | H. Hao [28]            | Y  | Y  | U  | U  | U  | Y  | Y  | Y  | U  | 4     |
| 5  | H. Yang [45]           | U  | Y  | U  | Y  | U  | U  | Y  | Y  | Y  |      | 5     |
| 6  | H. Yu [49]             | U  | Y  | U  | Y  | U  | U  | Y  | Y  | Y  |      | 4     |
| 7  | K. Chen [39]           | U  | Y  | U  | Y  | U  | U  | Y  | Y  | Y  |      | 5     |
| 8  | K. Hou [29]            | U  | Y  | U  | Y  | U  | U  | Y  | Y  | Y  |      | 5     |
| 9  | K. R. Nalamolu [27]    | U  | Y  | U  | Y  | U  | U  | Y  | Y  | U  |      | 4     |
| 10 | L. Xu [33]             | U  | U  | Y  | U  | U  | U  | Y  | Y  | Y  |      | 4     |
| 11 | M. Han [40]            | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 4     |
| 12 | Q. Pan [43]            | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 4     |
| 13 | W. Wang [44]           | U  | Y  | U  | Y  | U  | U  | Y  | Y  | Y  |      | 5     |
| 14 | X. Huang [26]          | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 4     |
| 15 | X. Li [34]             | U  | Y  | U  | U  | U  | U  | Y  | Y  | Y  |      | 4     |
| 16 | X. Li [42]             | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 5     |
| 17 | X. Liu [35]            | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 4     |
| 18 | Y. AN [48]             | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 5     |
| 19 | Y. Ye [46]             | Y  | Y  | Y  | Y  | Y  | U  | Y  | Y  | Y  |      | 9     |
| 20 | Y. Zhao [30]           | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 4     |
| 21 | Y. Zhao [47]           | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 5     |
| 22 | Z. Hou [41]            | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 4     |
| 23 | Z. Pan [31]            | U  | Y  | U  | Y  | U  | U  | U  | Y  | Y  |      | 5     |
| 24 | Z. Zhang [37]          | U  | U  | U  | Y  | U  | U  | Y  | Y  | Y  |      | 4     |

①: Was the allocation sequence adequately generated and applied; ②: were the groups similar at baseline or were they adjusted for confounders in the analysis; ③: was the allocation adequately concealed; ④: was the allocation sequence adequately generated and applied; ⑤: were the groups similar at baseline or were they adjusted for confounders in the analysis; ⑥: were animals randomly housed during the experiment; ⑦: were incomplete outcome data adequately addressed; ⑧: were the animals randomly housed during the experiment; ⑨: were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment; ⑩: were reports of the study free of selective outcome reporting; U: uncertain.

30–200 nm. 2 studies [27, 39] did not report the size of its EVs (Table 1).

3.4. Study Quality. The score of study quality ranged from three to nine in a total of ten points. Of which, 2 studies [32, 36] got three points; 12 studies [26–28, 30, 33–35, 37, 40, 41, 43, 49] got four points; 8 studies [29, 31, 39, 42, 44, 45, 47, 48] got five points; 1 study [38] got six points; and 1 study [46] got nine points. Most studies lacked reliable randomization methods, binding methods, or allocation concealment. The methodological quality is concluded in Table 2.

3.5. Effectiveness

3.5.1. Cerebral Infarction Volume. Meta-analysis of 21 studies [26–29, 31–33, 35–41, 43–49] showed significant effects of MSCs-derived sEVs for decreasing the cerebral infarction volume compared with control group (n = 138, SMD: -3.76, 95% CI: -4.78 to -3.50, P < 0.00001; heterogeneity: $X^2 = 19.92$, df = 13 ($P = 0.10$, $I^2 = 35\%$) (Figure 3).

3.5.3. Neurological Impairment Score. Meta-analysis of 12 studies [28, 30–32, 34, 35, 37, 40, 42, 46, 48, 49] showed significant effects of MSCs-derived exosomes for decreasing the neurological impairment score compared with control group (n = 91, SMD: -2.11, 95% CI: -2.51 to -1.70, P < 0.00001; heterogeneity: $X^2 = 21.17$, df = 11 ($P = 0.03$, $I^2 = 48\%$) (Figure 4).

3.5.4. Brain Water Content. Meta-analysis of 4 studies [32, 35, 40, 43] showed significant effects of MSCs-derived sEVs for decreasing the brain water content compared with control group (n = 26, SMD: -2.45, 95% CI: -3.25 to -1.65, P < 0.00001; heterogeneity: $X^2 = 2.48$, df = 3 ($P = 0.48$, $I^2 = 0\%$) (Figure 5).

3.5.5. Caspase-3. Meta-analysis of 6 studies [26, 29, 31, 36, 39, 44] showed significant effects of MSCs-derived sEVs for reducing the level of caspase-3 compared with control
group \( n = 39 \), SMD: \(-5.40\), 95% CI: \(-6.55 \) to \(-4.24\), \( P < 0.00001 \); heterogeneity: \( X^2 = 9.00, df = 5 \ (P = 0.11), I^2 = 44\% \) (Figure 6).

3.5.6. TNF-α. Meta-analysis of 8 studies [32, 33, 37, 39, 42, 44, 45, 49] showed significant effects of MSCs-derived sEVs for reducing the expression of proinflammatory factor TNF-α compared with control group \( n = 48 \), SMD: \(-2.60\), 95% CI: \(-3.23 \) to \(-1.96\), \( P < 0.00001 \); heterogeneity: \( X^2 = 11.35, df = 7 \ (P = 0.12), I^2 = 38\% \) (Figure 7).

3.5.7. IL-1β. Meta-analysis of 7 studies [35, 37, 39, 42, 45, 47, 49] showed significant effects of MSCs-derived sEVs for reducing the expression of proinflammatory factor IL-1β compared with control group \( n = 39 \), SMD: \(-2.57\), 95% CI: \(-3.27 \) to \(-1.86\), \( P < 0.00001 \); heterogeneity: \( X^2 = 10.41, df = 6 \ (P = 0.11), I^2 = 42\% \) (Figure 8).

3.5.8. IL-6. Meta-analysis of 8 studies [32, 33, 37, 42, 44, 45, 47, 49] showed significant effects of MSCs-derived sEVs for reducing the expression of proinflammatory factor IL-6 compared with control group \( n = 45 \), SMD: \(-2.28\), 95% CI: \(-2.90 \) to \(-1.65\), \( P < 0.00001 \); heterogeneity: \( X^2 = 12.31, df = 7 \ (P = 0.09), I^2 = 43\% \) (Figure 9).

3.6. Publication Bias Analysis. The publication bias was analyzed by funnel plot, and the volume of cerebral infarction was selected to draw the funnel plot. The funnel plot of
Ischemic stroke remains a leading cause of mortality and disability worldwide, placing a huge economic burden on society. During ischemic cerebrovascular events, the most crucial goal for treatment is to restore blood flow to the cerebral infarction volume, as shown in Figure 10, is uneven in distribution and has a certain publication bias, which may be due to the inclination of positive publication and ignoring negative results and the lack of search of literature other than Chinese and English. Publication bias could only increase unreliability.

4. Discussion
ischemic penumbra. However, the restoration of blood flow will cause reperfusion injury, which eventually leads to neuronal death in ischemic penumbra via apoptosis and necrosis. Apoptosis is one of the major mechanisms of cell death during cerebral ischemia and reperfusion injury, which is the main cause of neuronal death in the central nervous system during cerebral ischemia [26]. Future focus could be directed towards inhibiting neuronal apoptosis to recover neuronal structure and function of rats after CIRI [31]. Additionally, inflammation also serves an important role during cerebral ischemia-reperfusion injury [28]. Despite efforts to reduce cerebral ischemia/reperfusion injury, an
An ideal therapeutic approach for clinical neuroprotection against ischemia/reperfusion injury is still lacking.

Previous studies suggested that cell-based therapy using MSCs may not only be an effective reparative treatment but also a brain-protective therapy that improves neurological recovery [53–55]. Recently, exosomes derived from MSCs have been found to carry various kinds of mediators, miRNAs, and proteins, which can mediate the function of MSCs [56–58]. There is growing evidence that MSCs-derived exosomes can play important roles in repairing brain-injured tissues [26]. However, we were unable to locate any systematic reviews or reviewers regarding the attenuation of CIRI by exosomes derived from MSCs in PUBMED or Web of Science. There are 24 control trials [26–49] published from 2016 to 2022 providing new evidence. Thus, an updated meta-analysis is essential. This meta-analysis is based on 24 controlled preclinical trials to demonstrate that MSCs-derived sEVs could significantly inhibit CIRI, in terms of cerebral infarct volume, apoptosis rate, neurological impairment scores, brain water content, and neuroinflammation.

Mesenchymal stem cells (MSCs) have been widely used in the experimental or clinical treatment of various ischemic diseases, but the therapeutic efficacy of MSCs on CIRI requires more research. The ethical issue is the main factor hindering advancement in clinical research. Human umbilical cord MSCs (hUMSCs), autologous adipose-derived MSCs, and autologous bone marrow-derived MSCs (BMSCs) are associated with minimal ethical controversy compared to other stem cells. Among the 24 studies included in this meta-analysis, the sources of MSCs were bone marrow MSCs in 12 studies [29, 30, 34–36, 38, 40, 42, 43, 45, 47, 49], human umbilical cord MSCs in 7 studies [27, 31, 32, 37, 44, 46, 48], and adipose MSCs in 5 studies [26, 28, 33, 39, 41]. Relative to human BMSCs, hUMSCs are more readily obtained, exhibit superior viability, are compatible with therapeutic methods featuring higher levels of patient acceptability and compliance, and are not susceptible to immune-mediated graft rejection [59, 60]. Stroke occurs frequently between the ages of 45 and 65, and there is an autologous bone marrow aging problem. In clinical research, it can minimize pain during bone marrow extraction and enhance volunteer compliance [37].

The sEVs derived from MSCs could mitigate nerve injury after cerebral I/R confirmed by some studies. Cheng et al. demonstrated that MSCs-derived exosomes attenuate ischemia-reperfusion brain injury and inhibit microglia apoptosis might via exosomal miR-26a-5p mediated suppression of CDK6 [36]. Furthermore, Li et al. drew a conclusion that exosomal miR-26b-5p could mitigate nerve injury after cerebral I/R by targeting CH25H and inactivating the TLR pathway [32]. Hou et al. also found that negative regulation of PTEN and activation of Akt mediated the effects of miR-29b-3p on the amelioration of brain injury caused by hypoxic ischemia [29]. Subsequently, they found out that miR-29b-3p delivered in exosomes from BMSCs accelerated angiogenesis of BMECs and hindered neuronal apoptosis after ischemic stroke via targeting PTEN and activating the Akt signaling pathway [29].

In the current analysis, the quality of included studies was considered as moderate, which ranged from three to nine out of a ten. The main drop points are that no study reported the allocation scheme concealment, whether the participants and the investigator adopted the blind method and whether the blind method was applied to the result evaluation. Secondly, only 2 studies [28, 46] (8.33%) reported the random allocation method. Therefore, future research should pay more attention to the application of the blind method in experimental design, and at the same time, the
specific experimental implementation details should be reported comprehensively, so as to improve the repeatability and reliability of animal experimental results.

Various pharmacological agents have been shown to reduce CIRI in animal models. However, lack of neuroprotectant has been routinely utilized for clinical CIRI so far. One of the major results of this meta-analysis was that MSCs-derived sEVs significantly alleviated neurological impairment scores, reduced the volume of cerebral infarction and brain water content, and attenuated neuronal apoptosis in mice or rats MCAO model, and heterogeneity was not evident, indicating that sEVs showed consistent therapeutic potential in inhibiting CIRI and alleviating neuron damage. Furthermore, the main pathways of apoptosis include extracellular signal-triggered caspase activation and intracellular apoptotic enzyme release from mitochondria, which activate caspase [61]. As we can see, caspase plays an important role in apoptosis and is involved in the common pathway of various apoptotic signals. Among them, caspase-3 is the most important terminal cleavage enzyme in the process of cell apoptosis [62]. Our results provide evidence that MSCs-derived sEVs reduced the level of caspase-3 with no obvious heterogeneity observed, which confirmed that sEVs can suppress neuron apoptosis via caspase-3 pathway.

Acute ischemic stroke has been demonstrated to induce the inflammatory response accompanied by a significant increase in the expression levels of inflammatory and proinflammatory cytokines markers [63]. Microglia release proinflammatory cytokines such as IL-1β, IL-6, and TNF-α in the acute phase of ischemic stroke, impeding postinjury neural regeneration and producing poorer long-term neurological outcomes [64, 65]. Studies have proven that decreasing microglia-mediated neuroinflammation is beneficial during stroke recovery [65, 66]. The other of the major results of this meta-analysis was that MSCs-derived sEVs inhibited the expression of proinflammatory factors (TNF-α, IL-1β, IL-6) and attenuated microglia-mediated neuroinflammation after ischemic stroke and heterogeneity were not observed obviously. So, MSCs-derived sEVs can reduce CIRI by anti-inflammation.

Although results of this meta-analysis were supported by powerful proof, some limitations were worth noting. First, due to the relatively short number of trials, we were unable to conduct an in-depth metaregression analysis and subgroup analysis. Second, the neurological impairment scores included in this study varied over time, and the long-term follow-up effect could not be further analyzed, so that the long-term effect was not supported by corresponding evidence. Third, the parameters we chose are insufficient to demonstrate the full range of exosomes functions. Fourth, we acknowledge that the study did not retrieve unpublished literature and was limited to Chinese and English research, and the funnel chart suggests that there may be a certain publication bias, which could exaggerate the positive results. Fifth, most of the included studies did not report allocation concealment or blind method, which has a certain risk of bias. Sixth, because some data cannot be obtained directly in research, we measured the numerical values from the graphs, which led to possible deviations between estimated and actual statistical data. Finally, 24 studies included in this meta-analysis all used healthy adult rats or mice that fail to account for preexisting stroke risk factors such as hypertension, obesity, diabetes, sex, and aging. Generally, various factors exert important effects on the outcome in this stroke model, necessitating further research.

5. Conclusions
MSCs-derived sEVs could effectively attenuate CIRI in vivo and inhibit microglia-mediated neuroinflammation, which might be regarded as a novel insight for cerebral ischemic stroke. The preclinical results are encouraging for preparing and using feasibility studies in humans. However, more in-depth research is needed in the future to validate the therapeutic safety, in order to draw a more reliable and persuasive conclusion.

Data Availability
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Conflicts of Interest
The authors confirm that this article’s content has no conflict of interest.

Acknowledgments
The work was supported by Hainan Province Clinical Medical Center and Applied Basic Research Program (Natural Science Field) High-level Talent Project (2019RC386).

Supplementary Materials
File S1: PRISMA checklist for this systematic review. File S2: Database retrieval strategy of PubMed. (Supplementary Materials)

References
[1] D. Sacks, B. Baxter, B. Campbell et al., “Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke,” International Journal of Stroke, vol. 13, no. 6, pp. 612–632, 2018.
[2] L. Dereux and T. H. Cho, “Mechanical thrombectomy in acute ischemic stroke,” Revue Neurologique (Paris), vol. 173, no. 3, pp. 106–113, 2017.
[3] H. Amani, E. Mostafavi, M. R. Alebouyeh et al., “Would colloidal gold nanocarriers present an effective diagnosis or treatment for ischemic stroke?,” International Journal of Nanomedicine, vol. 14, pp. 8013–8031, 2019.
[4] Q. Yuan, Y. Yuan, Y. Zheng et al., “Anti-cerebral ischemia reperfusion injury of polysaccharides: a review of the mechanisms,” Biomedicine & Pharmacotherapy, vol. 137, article 111303, 2021.
[5] M. F. Pittenger, D. E. Discher, B. M. Péault, D. G. Phinney, J. M. Hare, and A. I. Caplan, “Mesenchymal stem cell
perspective: cell biology to clinical progress,” *NPJ Regenerative Medicine*, vol. 4, no. 1, p. 22, 2019.

[6] Y. Zhang, L. Ma, Y. Su et al., “Hypoxia conditioning enhances neuroprotective effects of aged human bone marrow mesenchymal stem-cell-derivied conditioned medium against cerebral ischemia in vitro,” *Brain Research*, vol. 1725, article 146432, 2019.

[7] L. X. Pang, W. W. Cai, Q. Li et al., “Bone marrow-derived mesenchymal stem cells attenuate myocardial ischemia-reperfusion injury via upregulation of splenic regulatory T cells,” *BMC Cardiovascular Disorders*, vol. 21, no. 1, p. 215, 2021.

[8] Q. Zhang, X. Liu, C. Piao et al., “Effect of conditioned medium from adipose derived mesenchymal stem cells on endoplasmic reticulum stress and lipid metabolism after hepatic ischemia reperfusion injury and hepatectomy in swine,” *Life Sciences*, vol. 289, article 120212, 2022.

[9] D. Kong, Y. Hu, X. Li et al., “IL-37 gene modification enhances the protective effects of mesenchymal stromal cells on intestinal ischemia reperfusion injury,” *Stem Cells International*, vol. 2020, Article ID 8883636, 12 pages, 2020.

[10] D. Gu, X. Zou, G. Ju, G. Zhang, E. Bao, and Y. Zhu, “Mesenchymal stromal cells derived extracellular vesicles ameliorate acute renal ischemia reperfusion injury by inhibition of mitochondrial fission through MIR-30,” *Stem Cells International*, vol. 2016, Article ID 2093940, 12 pages, 2016.

[11] V. Miceli, A. Bertani, C. M. Chinnici et al., “Conditioned medium from human amnion-derived mesenchymal stromal/stem cells attenuating the effects of cold ischemia-reperfusion injury in an in vitro model using human alveolar epithelial cells,” *International Journal of Molecular Sciences*, vol. 22, no. 2, p. 510, 2021.

[12] Y. S. Zhao, H. F. Zhao, X. L. Wang, Y. L. Cao, F. Q. Zhang, and J. Zhang, “Bone marrow mesenchymal stem cells effects on retinal ischemia-reperfusion injury after subretinal transplantation,” *Journal of Clinical Reconstructive Tissue Engineering Research*, vol. 12, pp. 9261–9264, 2008.

[13] Y. Fujita, H. Nakai, S. Masuda et al., “Intravenous injection of adult human bone marrow mesenchymal stromal cells attenuates spinal cord ischemia reperfusion injury in a mouse aortic arch cross clamping model,” *Circulation*, vol. 138, Supplement 1, 2018.

[14] V. T. Nooshabadi, S. Mardpour, A. Yousefi-Ahmadipour et al., “The extracellular vesicles-derived from mesenchymal stromal cells: a new therapeutic option in regenerative medicine,” *Journal of Cellular Biochemistry*, vol. 119, no. 10, pp. 8048–8073, 2018.

[15] R. Xu, A. Rai, M. Chen, W. Suwakulsiri, D. W. Greening, and R. J. Simpson, “Extracellular vesicles in cancer – implications for future improvements in cancer care,” *Nature Reviews. Clinical Oncology*, vol. 15, no. 10, pp. 617–638, 2018.

[16] G. van Niel, G. D’Angelo, and G. Raposo, “Shedding light on the cell biology of extracellular vesicles,” *Nature Reviews. Molecular Cell Biology*, vol. 19, no. 4, pp. 213–228, 2018.

[17] L. Mashouri, H. Yousefi, A. R. Aref, A. M. Ahadi, F. Molaei, and S. K. Alahari, “Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance,” *Molecular Cancer*, vol. 18, no. 1, p. 75, 2019.

[18] S. Mathivanan, H. Ji, and R. J. Simpson, “Exosomes: extracellular organelles important in intercellular communication,” *Journal of Proteomics*, vol. 73, no. 10, pp. 1907–1920, 2010.

[19] E. D’Asti, D. Garnier, T. H. Lee, L. Montermini, B. Meehan, and J. Rak, “Oncogenic extracellular vesicles in brain tumor progression,” *Frontiers in Physiology*, vol. 3, p. 294, 2012.

[20] B. Liu, W. Zheng, L. Dai, S. Fu, and E. Shi, “Bone marrow mesenchymal stem cell derived exosomal miR-455-5p protects against spinal cord ischemia reperfusion injury,” *Tissue and Cell*, vol. 74, article 101678, 2022.

[21] C. Wang, G. Zhu, W. He et al., “BMSCs protect against renal ischemia-reperfusion injury by secreting exosomes loaded with miR-199a-5p that target BIP to inhibit endoplasmic reticulum stress at the very early reperfusion stages,” *The FASEB Journal*, vol. 33, no. 4, pp. 5440–5456, 2019.

[22] B. Yang, W. Duan, L. Wei et al., “Bone marrow mesenchymal stem cell-derived hepatocyte-like cell exosomes reduce hepatic ischemia/reperfusion injury by enhancing autophagy,” *Stem Cells and Development*, vol. 29, no. 6, pp. 372–379, 2020.

[23] J. K. Zhang, Z. Zhang, Z. A. Guo et al., “The BMSC-derived exosomal lncRNA Mir9-3hg suppresses cardiomyocyte ferroptosis in ischemia-reperfusion mice via the Pum2/PRDX6 axis,” *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 32, no. 2, pp. 515–527, 2022.

[24] J. W. Li, L. Wei, Z. Han, and Z. Chen, “Mesenchymal stromal cells-derived exosomes alleviate ischemia/reperfusion injury in mouse lung by transporting anti-apoptotic miR-21-5p,” *European Journal of Pharmacology*, vol. 852, pp. 68–76, 2019.

[25] A. Kalani, P. Chaturvedi, P. K. Kamat et al., “Curcumin-loaded embryonic stem cell exosomes restored neurovascular unit following ischemia-reperfusion injury,” *The International Journal of Biochemistry & Cell Biology*, vol. 79, pp. 360–369, 2016.

[26] X. Huang, J. Ding, Y. Li et al., “Exosomes derived from PEDF modified adipose-derived mesenchymal stem cells ameliorate cerebral ischemia-reperfusion injury by regulation of autophagy and apoptosis,” *Experimental Cell Research*, vol. 371, no. 1, pp. 269–277, 2018.

[27] K. R. Nalamolu, I. Venkatesh, A. Mohandass et al., “Exosomes treatment mitigates ischemic brain damage but does not improve post-stroke neurological outcome,” *Cellular Physiology and Biochemistry*, vol. 52, no. 6, pp. 1280–1291, 2019.

[28] H. Hao, F. Zhou, and D. Yu, “Effect of exosomes derived from adipose-derived mesenchymal stem cells on neuron apoptosis and inflammatory cytokines in a rat model of cerebral ischemia reperfusion,” *Journal of Army Medical University*, vol. 41, no. 17, 2019.

[29] K. Hou, G. Li, J. Zhao et al., “Retracted ARTICLE:Bone mesenchymal stem cell-derived exosomal microRNA-29b-3p prevents hypoxic-ischemic injury in rat brain by activating the PTEN-mediated Akt signaling pathway,” *Journal of Neuroinflammation*, vol. 17, no. 1, 2020.

[30] Y. Zhao, Y. Gan, G. Xu, G. Yin, and D. Liu, “MSCs-derived exosomes attenuate acute brain injury and inhibit microglial inflammation by reversing CysLT2-ERK1/2 mediated microglia M1 polarization,” *Neurochemical Research*, vol. 45, no. 5, pp. 1180–1190, 2020.

[31] Z. Pan, “Effects of exosomes derived from H-BDNF-MSCs on neurons apoptosis in rats cerebral ischemia-reperfusion injury,” *University of South China*, 2020.
Z. Hou, J. Chen, H. Yang, X. Hu, and F. Yang, “Exosomes-carried microRNA-26b-5p regulates microglia M1 polarization after cerebral ischemia/reperfusion,” *Cell Cycle*, vol. 19, no. 9, pp. 1022–1035, 2020.

L. Xu, H. Ji, Y. Jiang et al., “Exosomes derived from CIRC-Akap7-modified adipose-derived mesenchymal stem cells protect against cerebral ischemic injury,” *Frontiers in Cell and Developmental Biology*, vol. 8, 2020.

X. Li, Y. Zhang, Y. Wang et al., “Exosomes derived from CXCR4-overexpressing BMSC promoted activation of microvascular endothelial cells in cerebral ischemia/reperfusion injury,” *Neural Plasticity*, vol. 2020, Article ID 8814239, 13 pages, 2020.

X. Liu, M. Zhang, H. Liu et al., “Bone marrow mesenchymal stem cell-derived exosomes attenuate cerebral ischemia-reperfusion injury-induced neuroinflammation and pyroptosis by modulating microglia M1/M2 phenotypes,” *Experimental Neurology*, vol. 341, article 113700, 2021.

C. Cheng, X. Chen, Y. Wang et al., “MSCs-derived exosomes attenuate ischemia-reperfusion brain injury and inhibit microglia apoptosis might via exosomal miR-26a-5p mediated suppression of CDK6,” *Molecular Medicine*, vol. 27, no. 1, 2021.

Z. Zhang, X. Zou, R. Zhang et al., “Human umbilical cord mesenchymal stem cell-derived exosomal miR-146a-5p reduces microglial-mediated neuroinflammation via suppression of the IRAK1/TRAF6 signaling pathway after ischemic stroke,” *Aging*, vol. 13, no. 2, pp. 3060–3079, 2021.

B. Feng, L. Meng, L. Luan, Z. Fang, P. Zhao, and G. Zhao, “Upregulation of extracellular vesicles-encapsulated miR-132 released from mesenchymal stem cells attenuates ischemic neuronal injury by inhibiting Smad2/3-c-Jun pathway via ACVR2b suppression,” *Frontiers in Cell and Developmental Biology*, vol. 8, 2021.

K. Chen, C. Chen, C. G. Wallace et al., “Intravenous administration of xenogenic adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes markedly reduced brain infarct volume and preserved neurological function in rat after acute ischemic stroke,” *OncoTarget*, vol. 7, no. 46, pp. 74537–74556, 2016.

M. Han, Y. Cao, H. Xue et al., “CpG Neuroprotective effect of mesenchymal stromal cell-derived extracellular vesicles against cerebral ischemia-reperfusion-induced neuronal functional injury: a pivotal role for AMPK and JAK2/STAT3/NF-κB pathway modulation,” *Drug Design Development and Therapy*, vol. 14, pp. 2865–2876, 2020.

Z. Hou, J. Chen, H. Yang, X. Hu, and F. Yang, “microRNA-26a shuttled by extracellular vesicles secreted from adipose-derived mesenchymal stem cells reduce neuronal damage through KLF9-mediated regulation of TRAF2/KLF2 axis,” *Adipocyte*, vol. 10, no. 1, pp. 378–393, 2021.

X. Li, T. Bi, and S. Yang, "Exosomal microRNA-150-5p from bone marrow mesenchymal stromal cells mitigates cerebral ischemia/reperfusion injury via targeting toll-like receptor 5," *Bioengineered*, vol. 13, no. 2, pp. 3030–3043, 2022.

Q. Pan, X. Kuang, S. Cai et al., “miR-132-3p priming enhances the effects of mesenchymal stromal cell-derived exosomes on ameliorating brain ischemic injury,” *Stem Cell Research and Therapy*, vol. 11, no. 1, p. 260, 2020.

W. Wang, Z. Ji, C. Yuan, and Y. Yang, “Mechanism of human umbilical cord mesenchymal stem cells derived-extracellular vesicle in cerebral ischemia-reperfusion injury,” *Neurochemical Research*, vol. 46, no. 3, pp. 455–467, 2021.

H. Yang and J. Chen, “Bone marrow mesenchymal stem cell-derived exosomes carrying long noncoding RNA ZFAS1 alleviate oxidative stress and inflammation in ischemic stroke by inhibiting microRNA-15a-5p,” *Metabolic Brain Disease*, 2022.

Y. Ye, Z. Chang, P. Wang et al., “Infarct-preconditioning exosomes of umbilical cord mesenchymal stem cells promoted vascular remodeling and neurological recovery after stroke in rats,” *Stem Cell Research & Therapy*, vol. 13, no. 1, p. 378, 2022.

Y. Zhao, Y. Gan, G. Xu, K. Hua, and D. Liu, “Exosomes from MSCs overexpressing microRNA-223-3p attenuate cerebral ischemia through inhibiting microglial M1 polarization mediated inflammation,” *Life Sciences*, vol. 260, p. 118403, 2020.

F. An, The restoration effect of the exosomes derived from high-expression BDNF MSCs in MCAO rats, University of South China, 2019.

H. Yu, Effects of bone marrow mesenchymal stem cells-derived miR-30c-5p on polarization of microglia in cerebral ischemia reperfusion and its mechanisms, Wuhan University, 2020.

J. Liu, T. Chen, P. Lei, X. Tang, and P. Huang, “Exosomes released by bone marrow mesenchymal stem cells attenuate lung injury induced by intestinal ischemia reperfusion via the TLR4/NF-κB pathway,” *International Journal of Medical Sciences*, vol. 16, no. 9, pp. 1238–1244, 2019.

C. Théry, K. W. Witwer, E. Aikawa et al., “Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines,” *J Extracellular Vesicles*, vol. 7, no. 1, article 1535750, 2018.

C. R. Hooijmans, M. M. Rovers, R. B. de Vries, M. Leenaars, M. Riskes-Hoitinga, and M. W. Langendam, “SYRCLE’s risk of bias tool for animal studies,” *BMC Medical Research Methodology*, vol. 14, no. 1, p. 43, 2014.

Y. K. Choi, E. Urnukhsaikhan, H. H. Yoon, Y. K. Seo, and J. K. Park, “Effect of human mesenchymal stem cell transplantation on cerebral ischemic volume-controlled photothermbolective mouse model,” *Biotechnology Journal*, vol. 11, no. 11, pp. 1397–1404, 2016.

H. Nakamura, Y. Sasaki, M. Sasaki et al., “Elevated brain derived neurotrophic factor levels in plasma reflect in vivo functional viability of infused mesenchymal stem cells for stroke in rats,” *Journal of Neurosurgical Sciences*, vol. 63, no. 1, pp. 42–49, 2019.

H. S. Jung, S. Y. Jeong, J. Yang et al., “Neuroprotective effect of mesenchymal stem cell through complement component 3 downregulation after transient focal cerebral ischemia in mice,” *Neuroscience Letters*, vol. 633, pp. 227–234, 2016.

D. Zhang, H. Lee, Z. Zhu, J. K. Minhas, and Y. Jin, “Enrichment of selective microRNAs in exosomes and delivery of exosomal miRNAs in vitro and in vivo,” *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 312, no. 1, pp. L110–L121, 2017.

F. H. Shamili, H. R. Bayegi, Z. Salmasi et al., “Exosomes derived from TRAIL-engineered mesenchymal stem cells with effective anti-tumor activity in a mouse melanoma model,” *International Journal of Pharmaceutics*, vol. 549, no. 1-2, pp. 218–229, 2018.

L. Bai, H. Shao, H. Wang et al., “Effects of mesenchymal stem cell-derived exosomes on experimental autoimmune uveitis,” *Scientific Reports*, vol. 7, no. 1, p. 4323, 2017.
D. L. Troyer and M. L. Weiss, “Wharton’s jelly-derived cells are a primitive stromal cell population,” Stem Cells, vol. 26, no. 3, pp. 591–599, 2008.

R. Friedman, M. Betancur, L. Boissel, H. Tuncer, C. Cetrulo, and H. Klingemann, “ Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation,” Biology of Blood and Marrow Transplantation, vol. 13, no. 12, pp. 1477–1486, 2007.

H. Zhu, X. Zheng, H. Feng et al., “Role of coflin-1 in arsenic trioxide-induced apoptosis of NB4-R1 cells,” Molecular Medicine Reports, vol. 22, no. 6, pp. 4645–4654, 2020.

Y. L. Sun, W. Q. Jiang, Q. Y. Luo et al., “A novel Bcl-2 inhibitor, BM-1197, induces apoptosis in malignant lymphoma cells through the endogenous apoptotic pathway,” BMC Cancer, vol. 20, no. 1, 2020.

L. L. Zeng, Y. T. Wang, J. R. Liu et al., “Pro-inflammatory cytokine network in peripheral inflammation response to cerebral ischemia,” Neuroscience Letters, vol. 548, pp. 4–9, 2013.

Y. K. Xie, J. H. Peng, J. W. Pang et al., “Biglycan regulates neuroinflammation by promoting M1 microglial activation in early brain injury after experimental subarachnoid hemorrhage,” Journal of Neurochemistry, vol. 152, no. 3, pp. 368–380, 2020.

Y. Y. Ma, J. X. Wang, Y. T. Wang, and G. Y. Yang, “The biphasic function of microglia in ischemic stroke,” Progress in Neurobiology, vol. 157, pp. 247–272, 2017.

X. M. Hu, R. K. Leak, Y. J. Shi et al., “Microglial and macrophage polarization–new prospects for brain repair,” Nature Reviews Neurology, vol. 11, no. 1, pp. 56–64, 2015.