Effects of extracellular vesicles for ischemic stroke: A meta-analysis of preclinical studies

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Abstract. Ischemic stroke is a common occurrence worldwide, posing a severe threat to human health and leading to negative financial impacts. Currently available treatments still have numerous limitations. As research progresses, extracellular vesicles are being found to have therapeutic potential in ischemic stroke. In the present study, the literature on extracellular vesicle therapy in animal studies of ischemic stroke was screened by searching databases, including PubMed, Embase, Medline, Web of Science and the Cochrane Library. The main outcomes of the present study were the neurological function score, apoptotic rate and infarct volumes. The secondary outcomes were pro-inflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6. The study quality was assessed using the CAMARADES Checklist. Subgroup analyses were performed to evaluate factors influencing extracellular vesicle therapy. Review Man3ager5.3 was used for data analysis. A total of 20 relevant articles were included in the present meta-analysis. The comprehensive analysis revealed that extracellular vesicles exerted a significant beneficial effect on neurobehavioral function, reducing the infarct volume and decreasing the apoptotic rate. Moreover, extracellular vesicles were found to promote nerve recovery by inhibiting pro-inflammatory factors (TNF-α, IL-1β and IL-6). On the whole, the present meta-analysis examined the combined effects of extracellular vesicles on nerve function, infarct volume, apoptosis and inflammation, which provides a foundation for the clinical study of extracellular vesicles.

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Introduction

Ischemic stroke is the leading cause of mortality and acquired physical disability worldwide. Ischemic stroke leads to the ischemic, anoxic necrosis of brain tissue due to insufficient cerebral blood supply, followed by the rapid occurrence of defects in the functions of corresponding nerves (1). It is characterized by a high incidence, disability, recurrence and a high mortality rate, accounting for 44 million physical disabilities, and 5.5 million related deaths worldwide annually (2). The current treatment of ischemic stroke focuses on rapid reperfusion with intravenous thrombolysis and endovascular thrombectomy (3); however, due to the limitations of a strict time window, damage to brain cells is irreversible, and some patients will have long-term disability after suffering a stroke (4).

Stem cell therapies have emerged as a potential therapeutic strategy for stroke in recent decades. Stem cells are initial and unspecialized cells that have the ability to self-renew, with high proliferative and multidirectional differentiation abilities; these cells include embryonic, mesenchymal and hematopoietic stem cells (5). The research focusing on stem cell therapy in ischemic stroke in animal models or clinical studies is rapidly progressing. There is evidence to suggest that stem cell therapies can repair damaged brain tissue and improve neurological function through multiple mechanisms, which includes the modulation of the immune response promotion of endogenous neural cells, angiogenesis and neuro-regeneration (6). However, stem cell therapies have also been found to be associated with certain risks, such as allogeneic cell transplantation, the development of infection and tumor formation (7).

A logical extension of stem cell therapies is the direct employment of solely extracellular vesicles as a treatment modality (8). The therapeutic benefit of stem cell-derived extracellular vesicles has been previously analyzed in ischemic stroke (9). Extracellular vesicles, including exosomes, microvesicles and apoptotic bodies, are membrane vesicles that are between 40-1,000 nm in diameter and are widely present in various bodily fluids. They can carry a variety of active molecules, such as proteins, lipids, messenger RNAs, microRNAs (miRNAs or miRs) and non-coding RNAs (10). Studies have shown that the content of exosomes, and the

variety of active molecules they carry, can impact the post transcriptional regulation of numerous genes. Hence, extracellular vesicles play a critical role in communications between cells. As natural delivery vehicles, they may be used for delivery across natural barriers, such as the blood-brain barrier. In addition, they avoid endogenous tumorigenicity due to less or no immunogenicity and tumorigenicity (9). Therefore, extracellular vesicles are expected to become a promising treatment strategy for ischemic stroke (10).

A plethora of preclinical studies on ischemic stroke models using extracellular vesicles have been conducted to explore their beneficial effects (9). However, there are only a limited number of trials using extracellular vesicles for the treatment of ischemic stroke in the database of clinical trials (6). To provide pre-clinical evidence for further and larger scale studies on extracellular vesicles in the treatment of ischemic stroke and promote the clinical applications of such vesicles in ischemic stroke, the present study performed a meta-analysis of animal studies.

Materials and methods

Search strategy. A search for relevant literature was performed using the following databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Embase (https://www.embase.com), Medline (https://www.nlm.nih.gov/medline/medline_home.html), Web of Science (https://www.webofscience.com) and the Cochrane Library (https://www.cochranelibrary.com/). The full search strategy was based on the following search terms: ('extracellular vesicles' or 'EVs') and ('cerebral ischemia' or 'ischemia stroke' or 'brain infarct' or 'cerebral infarct'). All publications were required to be published in English until May 2023. The reference list of the selected articles was independently be screened to identify additional studies excluded in the initial search. The present meta-analysis was registered in the PROSPERO database (https://www.crd.york.ac.uk/prospero/) under the registration number CRD42023442677.

Inclusion and exclusion criteria. The inclusion criteria for the study were as follows: i) Pre-clinical middle cerebral artery occlusion (MCAO) animal models, including mice or rats of any age or sex; ii) intervention: Cell-derived extracellular vesicles without modifications and transfection; iii) Comparisons: Vehicle, saline, phosphate-buffered saline, or no treatment; iv) Study designs: Randomized controlled experiments; v) outcome measures: Inclusion of neurological function score, apoptotic rate, infarct volumes, tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6; and vi) original full research articles. The exclusion criteria were the following: i) The study was not an animal study with ischemia stroke; ii) modified or transfected extracellular vesicles; iii) the intervention was a combination of extracellular vesicles and other therapy; iv) the study included *in vitro* experiments; v) the main information and evaluation indicators of the experiment were not reported; vi) the study was a review, editorial, case report, expert opinion, or letter; and vii) duplicate literature.

Data extraction. Of note, two authors independently extracted relevant data from the text and graphs. The data extraction sheet contained the following details from the included studies: i) Name of the first author and the year of publication; ii) number of animals per group for comparison; iii) intervention of experimental groups, control groups; iv) animal species, sex and weight; v) animal models: Method of induction of ischemia, type of anesthetic used, duration of reperfusion; vi) intervention of treatment: Time of administration, administration method, treatment dose, source of extracellular vesicles; vii) primary outcome measures: Neurological function score, infarct volumes and apoptotic rate; viii) secondary outcome measures: TNF-α, IL-1β and IL-6. Data were extracted data from charts with unavailable numerical values using GetData Graph Digitizer (version 2.26, https://apps.auto-meris.io/wpd/index.zh_CN.html) software (11).

Quality assessment. Two investigators independently assessed the quality of each eligible included study using the CAMARADES checklist for study quality. The CAMARADES checklist consists of 10 evaluation indicators, and the evaluation results are represented as 'Y', 'N' and 'U'. Any disagreements were resolved by discussion with a third author (12).

Statistical analysis. All statistical calculations and graphing were performed using Review Manager5.3 (The Cochrane Collaboration). The summaries of outcome measures were calculated using the standardized mean difference with 95% confidence intervals for continuous outcomes. A value of $P<0.05$ was considered to indicate a statistically significant difference. Statistical heterogeneity was evaluated using the I-square test and Q test. The analysis was combined using random effects model in the present study. In addition, subgroup analyses were conducted to explore the sources of between-study heterogeneity.

Results

Literature search results. A total of 2,996 potential studies were retrieved through the primary retrieval from the database, of which 749 articles were excluded due to reduplication. A total of 98 articles were included in the scope of review based on screening the titles and abstracts. Among these, 77 articles were excluded, due to the following reasons: i) The full text was not available; ii) *in vitro* studies; iii) modified or transfected extracellular vesicles; iv) the relevant results were incomplete. Ultimately, 20 studies were selected for the present meta-analysis. The specific flow chart for the literature search is presented in Fig. 1.

Characteristics of included studies. There were 20 studies were included in the meta-analysis (13-32). All articles were published between 2015 and 2023. Among the included studies, seven studies were performed using rats, and 13 studies were conducted on mice. The MCAO animal model to induce ischemic stroke was used in all the studies. The ischemia/reperfusion (I/R) model was established with filament insertion, thread loop blockage or microbipolar coagulation. Of the 20 preclinical animal studies, 10 of these used extracellular vesicles originating from bone marrow-derived mesenchymal stem cells, three studies used M2 microglial cell-derived extracellular vesicles, two studies used adipose

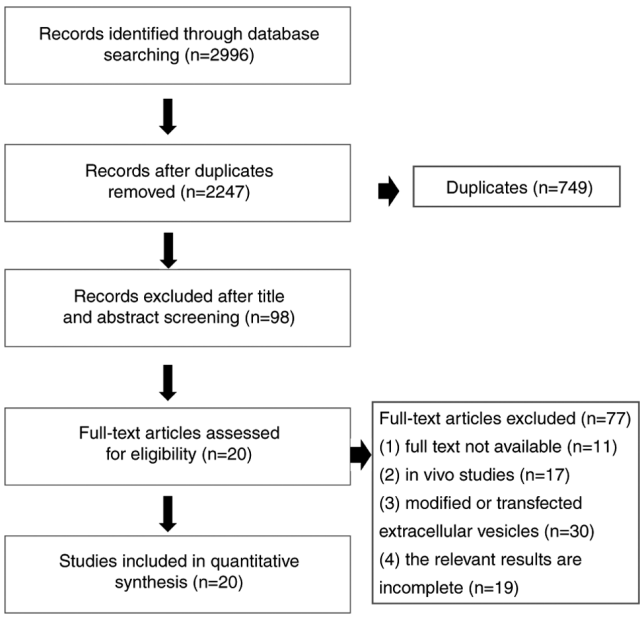

Figure 1. Flow chart of study selection.

stem cell-derived extracellular vesicles, and one study used extracellular vesicles derived from human umbilical cord perivascular cells, human placental mesenchymal cells, dental pulp stem cells, neural progenitor cells and astrocyte cells.

The route of extracellular vesicle administration was via tail vein injection in 11 studies, lateral ventricle injection in three studies, intravenous administration in five studies and nasal injection in one study. A variety of miRNA types have been researched in these studies, including miR-26a, miR-132, miR-124, miR-206, miR-135a-5p and miR-23a-3p. The characteristics of the included articles are presented in Table SI.

Quality assessment. The quality score across the 20 studies ranged from 3 to 10. The most suitable criteria included peer-reviewed journals, appropriate animal models, statements of potential conflicts of interests and statements of compliance with ethics or animal welfare regulations. Moreover, the random allocation of animals and temperature control were reported in the majority of the included studies. However, only a small number of studies had reported the blinding methods of model establishing and outcome assessment. The further score details of the study quality are presented in Table I.

Neurological function score. A total of 17 studies investigated neurological function following ischemic stroke, of which five studies used the modified neurological severity score (mNSS) to assess neurological function. Compared with the control group, the experimental group with extracellular vesicles was found to exhibit a significant improvement in the neurological function score (SMD: -2.46; 95% CI: -3.61 to -1.32; heterogeneity: P<0.00001; I^2=95%) (Fig. 2).

Infarct volume. A total of 9 studies involving infarct volume were comprehensively analyzed. Compared with the control group, extracellular vesicles were shown to significantly reduce the infarct volume following ischemic stroke (SMD: -24.64; 95% CI: -30.46 to -18.81; heterogeneity: P<0.00001; I^2=96%) (Fig. 3).

Apoptotic rate. A total of five studies reported the apoptotic rate in the MCAO animal model. A comprehensive analysis revealed that extracellular vesicles significantly decreased the apoptotic rate compared with the control group (SMD: -26.73; 95% CI: -31.92 to -21.53; heterogeneity: P=0.001; I^2=78%) (Fig. 4).

Secondary outcome. Some of the included studies evaluated the anti-inflammatory effects of external vesicles by measuring the levels and expression of inflammatory factors. A meta-analysis of four studies demonstrated that extracellular vesicles significantly decreased the level of IL-1β compared with the control group (SMD: -4.01; 95% CI: -6.52 to -1.50; heterogeneity: P=0.03; I^2=67%) (Fig. 5A). A comprehensive analysis of two studies revealed significant suppressive effects of extracellular vesicles on the level of IL-6 (SMD: -8.82; 95% CI: -17.71 to 0.06; heterogeneity: P=0.03; I^2=78%) (Fig. 5B). Additionally, a meta-analysis of two studies demonstrated that the level of TNF-α exhibited a significant decrease in the extracellular vesicle group (SMD: -1.27; 95% CI: -1.62 to -0.92; heterogeneity: P=0.49; I^2=0%) (Fig. 5C).

Publication bias analysis. The publication bias of the primary outcomes was examined using the funnel plot. The funnel plot for infarct volume and mNSS revealed significant asymmetry, suggesting a certain publication bias (Fig. 6). It was hypothesized that selection bias, implementation bias, measurement bias and reporting bias may all contribute to this asymmetry. Among the analysis research of neural function scores, the study from Dong *et al* (30) in 2021 lying outside of the funnels represent studies that have the highest publication bias. After reviewing the research process, it was found that the aforementioned study lacks a detailed description of the measurement process and analysis of the results of mNSS score. Possible differences in measurement time and method in the studies were considered to cause measurement bias. The studies, including Han *et al* (21) in 2020 and Liu *et al* (31) in 2022, have the highest publication bias in the analysis of studies on infarct volume. In the present analysis comparison, it was found that in the study of Han *et al* (21) and Liu *et al* (31), different software and methods were used to measure and calculate the infarct rates, and it was considered whether this would cause some measurement bias. Meanwhile, it was found that the specific measurement time of infarction volume was not reported in the study of Liu *et al* (31), and the possible difference in measurement time was also a major possible factor causing the bias. In addition, subgroup analysis was conducted from three aspects of the source of extracellular vesicles, the injection time or the injection method used, to identify the possible sources of publication bias.

Subgroup analyses. In the present study, subgroup analyses were conducted to explore the sources of heterogeneity based on different categories, including animal species, injection method and the sources of the extracellular vesicles. The results of the analyses demonstrated that the sources of the extracellular vesicles did not exhibit significant differences,

Table I. Quality evaluation of included studies.

ID	References	A	B	C	D	E	F	G	H	I	J
1	Seifali *et al* (13)	Y	Y	U	U	U	Y	U	U	Y	U
2	Wang *et al* (14)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3	Hu *et al* (15)	Y	Y	U	Y	U	Y	U	U	Y	Y
4	Gregorius *et al* (16)	Y	Y	Y	Y	U	Y	Y	Y	Y	Y
5	Dumbrava *et al* (17)	Y	Y	Y	Y	Y	Y	U	Y	Y	Y
6	Hu *et al* (18)	Y	U	U	Y	U	Y	U	Y	Y	Y
7	Li *et al* (19)	Y	Y	Y	Y	U	Y	U	Y	Y	Y
8	Tian *et al* (20)	Y	U	U	U	U	Y	U	U	Y	Y
9	Han *et al* (21)	Y	Y	U	Y	U	Y	Y	Y	Y	Y
10	Houa *et al* (22)	Y	Y	Y	U	U	Y	U	U	Y	Y
11	Doeppner *et al* (23)	Y	U	U	Y	U	Y	Y	Y	Y	Y
12	Heras-Romero *et al* (24)	Y	Y	Y	Y	Y	Y	U	Y	Y	Y
13	Li *et al* (25)	Y	Y	U	Y	U	Y	U	Y	Y	Y
14	Barzegarara *et al* (26)	Y	Y	U	U	U	Y	U	Y	Y	Y
15	Feng *et al* (27)	Y	Y	Y	U	U	Y	U	Y	Y	Y
16	Song *et al* (28)	Y	Y	U	Y	U	Y	U	Y	Y	Y
17	Liu *et al* (29)	Y	Y	U	U	U	Y	U	U	Y	Y
18	Dong *et al* (30)	Y	Y	Y	Y	U	Y	U	U	Y	Y
19	Liu *et al* (31)	Y	Y	Y	U	U	Y	U	Y	Y	Y
20	Xie *et al* (32)	Y	Y	U	U	U	Y	U	U	Y	Y

A, peer-reviewed journal; B, anesthetics with no intrinsic neuroprotective activity are used; C, temperature control; D, the random allocation of animals; E, calculation of sample size; F, appropriate animal models; G, blinded established model; H, blinded outcome assessment; I, statement of compliance with Ethics or animal welfare regulations; J, statement of potential conflict of interests; Y, yes; N, no; U, uncertain.

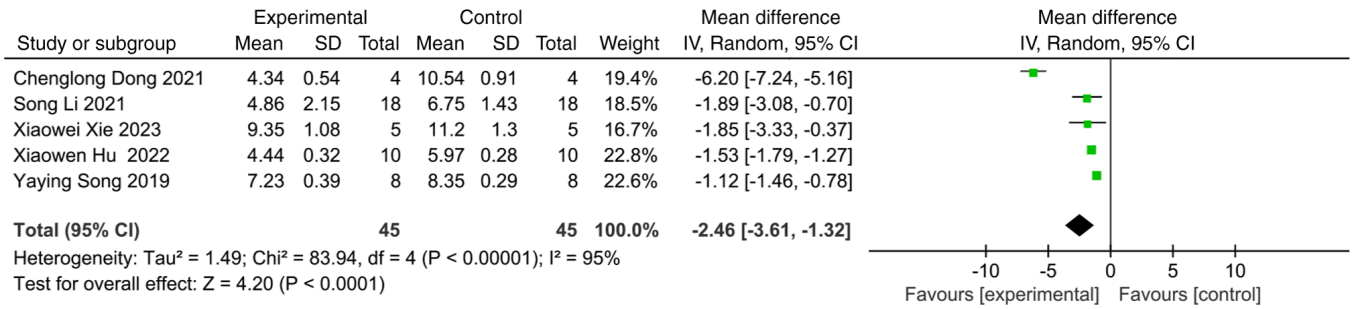

Figure 2. Forest plot of comparison of modified neurological severity score. SD, standardized difference; CI, confidence intervals; df, degrees of freedom; IV, inverse variance.

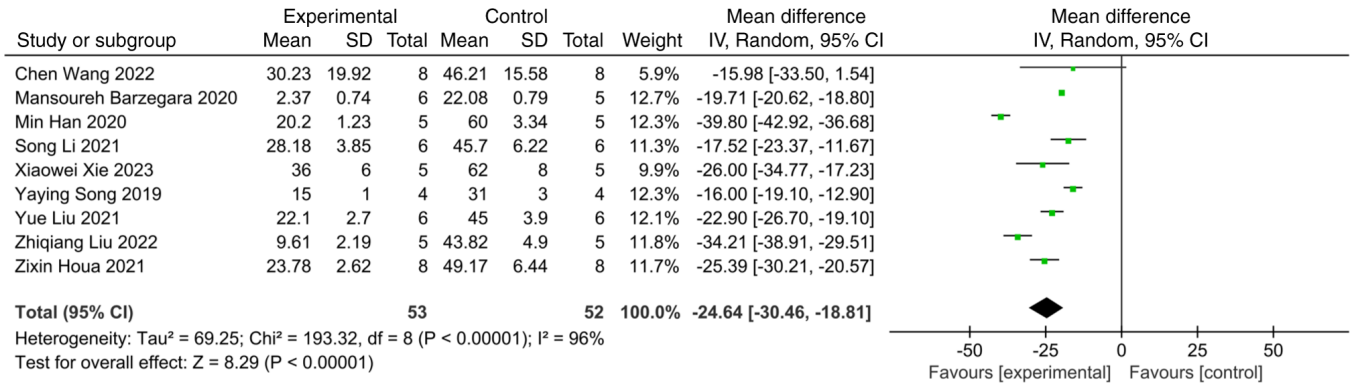

Figure 3. Forest plot of comparison of infarct volume. SD, standardized difference; CI, confidence intervals; df, degrees of freedom; IV, inverse variance.

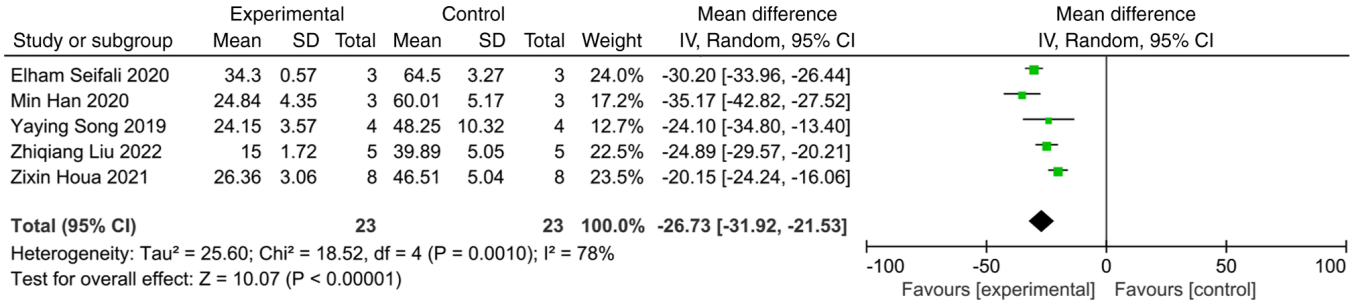

Figure 4. Forest plot of comparison of apoptotic rate. SD, standardized difference; CI, confidence intervals; df, degrees of freedom; IV, inverse variance.

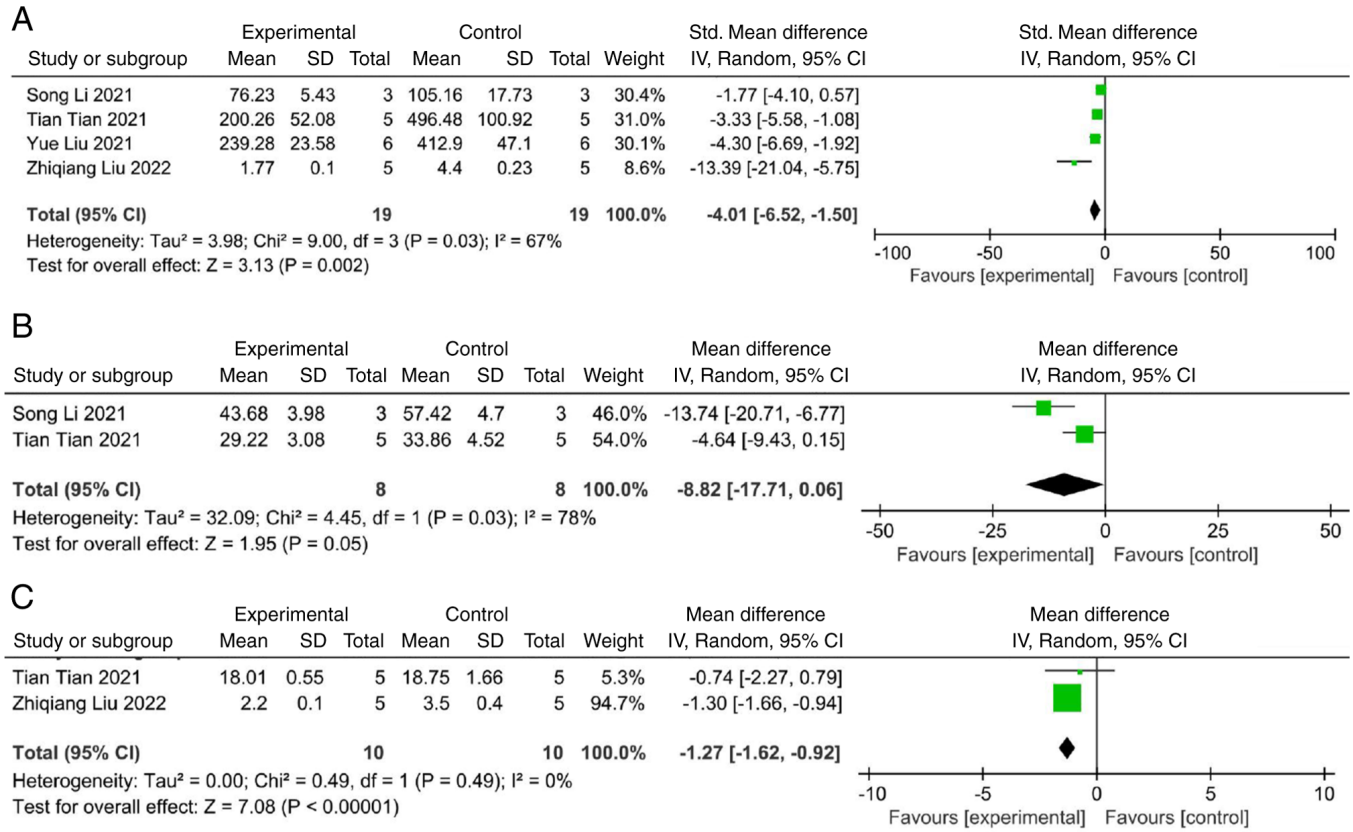

Figure 5. Forest plots of comparison of anti-inflammation factors included (A) interleukin 1β, (B) interleukin 6 and (C) tumor necrosis factor α. SD, standardized difference; CI, confidence intervals; df, degrees of freedom; IV, inverse variance.

and the animal species and injection method were the possible source of heterogeneity in the present study. All cell-derived extracellular vesicles improved the nerve function score (P=0.13; I^2=47.7%) (Fig. S1), decreasing the apoptotic rate (P=0.004; I^2=77.4%) (Fig. S2), and reducing the infarct volume (P=0.04; I^2=64%) (Fig. S3). In terms of neurological function score and apoptotic rate, the treatment effect of extracellular vesicles on mice compared with rats was as follows: mNSS, P<0.00001; I^2=98.7% (Fig. S4); apoptotic rate: P=0.006; I^2=86.6%) (Fig. S5); however, there was no significant difference in the therapeutic effect of animal species infarct volume (P<0.00001; I^2=96.7%) (Fig. S6). In addition, the effect size for tail vein injection was significantly larger than lateral ventricle injection in terms of neurological function score and apoptotic rate (mNSS: P<0.00001; I^2=98.7% (Fig. S7); apoptotic rate: P=0.66; I^2=0%) (Fig. S8). And the effect size of tail vein injection and lateral ventricle injection were larger than that of intravenous injection in infarct volume (P=0.05; I^2=66.4%) (Fig. S9). The detailed results of subgroup analyses are illustrated in Figs. S1-S9.

Discussion

The present meta-analysis of 20 studies with a total of 28 comparisons explored the overall effect of extracellular vesicles in the treatment of ischemic stroke. The evidence from included studies indicated that cell-derived extracellular vesicles significantly improved neurological function, decreased the infarct volume and inhibited apoptosis in the MACO model. Moreover, extracellular vesicles were also

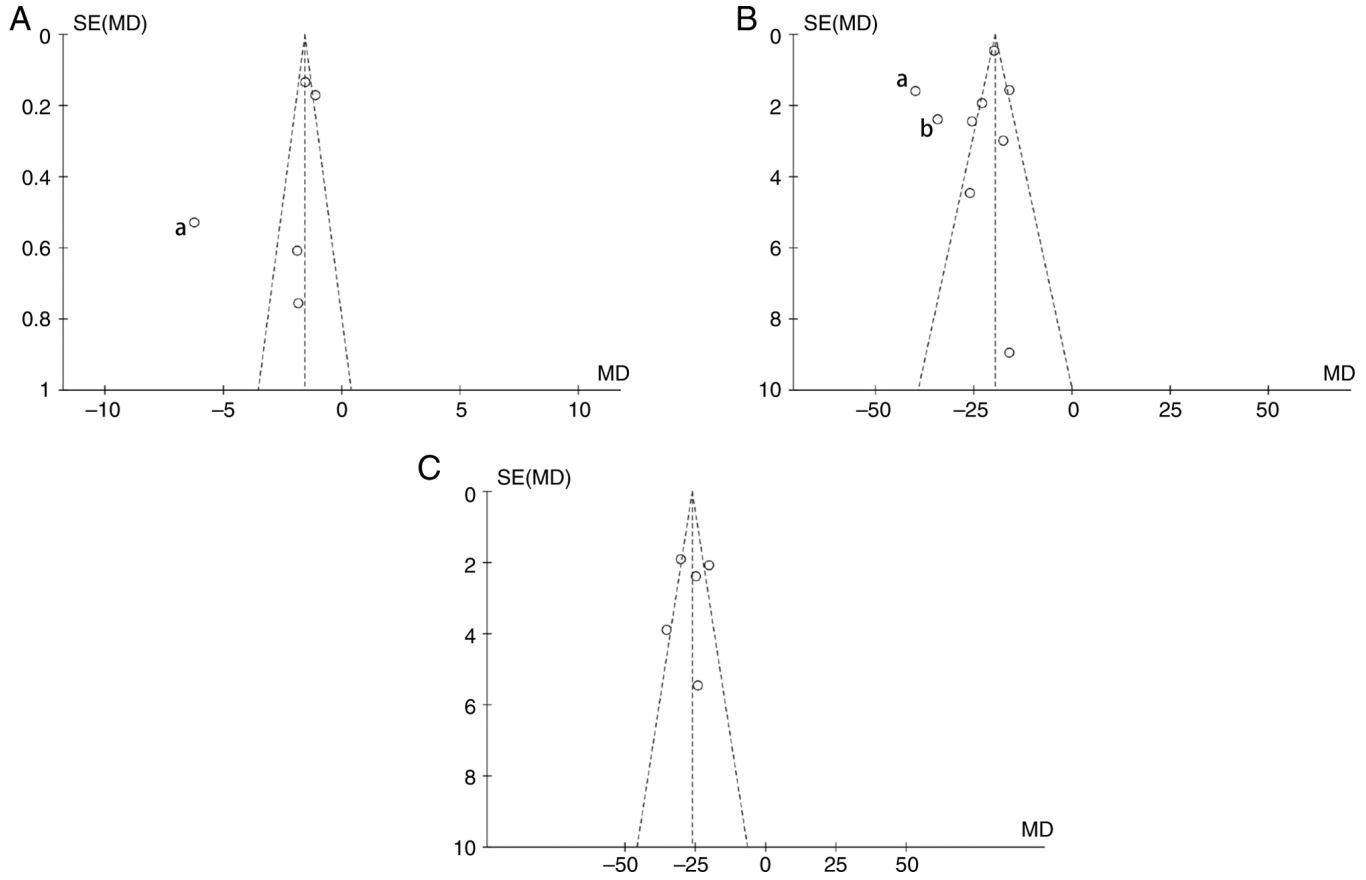

Figure 6. Funnel plots. (A) Modified neurological severity score. (B) Infarction volume. (C) Apoptotic rate. SE, standard error; MD, mean difference. Fig. 6A, point a: Dong *et al* (30); Fig. 6B, point a: Han *et al* (21); Fig. 6B, point b: Liu *et al* (31).

found to play a neuroprotective role by reducing neuroinflammation, confirmed by some studies. The present study used the CAMARADES checklist to evaluate the study quality, with a median of 7 and range from 3 to 10. Furthermore, attention needs to be paid to the calculation of sample size and the blind established model. The publication bias was found drawing the funnel plot. In summary, extracellular vesicle therapy is a promising research direction for ischemic stroke.

Previous research has demonstrated the positive efficacy of extracellular vesicles on infarct volume and neurological score in stroke models (33). Recently, cell-derived extracellular vesicles have been widely used in preclinical trials of ischemic stroke. There are studies that have focused on therapeutic efficacy and the mechanisms of extracellular vesicles and providing new evidence for further clinical trials. Hence, an updated meta-analysis is essential. The majority of previous meta-analyses on extracellular vesicles have examined infarct volume and neural function scores. By contrast, the present meta-analysis further evaluated the effects of extracellular vesicles on apoptosis and neuroinflammation.

In research investigating the application of extracellular vesicles in the treatment of ischemic stroke, the administration route of extracellular vesicles is still widely being explored. At present, the drug administration methods in extracellular vesicles in *in vivo* experiments are mainly divided into systemic administration and local administration; tail vein injection is widely used in the majority of preclinical trials (34). In the present meta-analysis, 11 included studies applied the tail vein injection, whereas three studies used lateral ventricle injection. In the subgroup analyses, the difference of effect size between tail vein injection and lateral ventricle injection was statistically significant.

Developing neuroprotective strategies remains a promising area of research in the treatment of stroke. There is evidence to demonstrate the neuroprotective potential of extracellular vesicles in preclinical ischemic stroke models (35). Extracellular vesicles can play a neuroprotective role to promote the recovery of nerve function and improve brain injury in ischemic stroke, as confirmed by the studies included in the present meta-analysis. For example, Han *et al* (21) demonstrated that mesenchymal stem cell-derived extracellular vesicles exerted neuroprotective effects, alleviating brain damage and reducing apoptosis during cerebral ischemia-reperfusion injury, which may be achieved by the regulation of the AMPK and JAK2/STAT3/NF-κB signaling pathways (21).

Cerebral vascular oxidative stress and apoptosis constitute a crucial pathological basis for ischemic stroke (4). There is increasing evidence to indicate that apoptosis has become a key target in the treatment of ischemic stroke. Han *et al* (21) found that mesenchymal stem cell-derived extracellular vesicles significantly reduced the number of apoptotic cells compared with the control group in MCAO (21). Furthermore, Feng *et al* (27) also demonstrated that miR-132-containing mesenchymal stem cell-derived extracellular vesicles decreased neuronal injury by inhibiting Acvr2b expression and the p-Smad2/c-Jun signaling pathway. Seifali *et al* (13)

reached the conclusion that extracellular vesicles have the potential to regulate apoptosis and improve neuronal recovery. Extracellular vesicle therapy following MCAO decreased neuronal apoptosis, enhanced neuronal density, reduced dark neurons and improved sensorimotor function (13). Cumulative evidence suggests that extracellular vesicles exert a positive effect by diminishing neuronal pathological injury and cell apoptosis following ischemic stroke.

Inflammation is widely involved in the pathogenesis of ischemic stroke. Following a stroke, dead cells release damage-associated molecular patterns, then recruit leukocytes to the brain and release inflammatory cytokines and chemokines. These stimulate an inflammatory response in microglia and astrocytes, which leads to secondary injury to the brain (36). It has been demonstrated that mesenchymal stem cell-derived exosomes inhibit microglial inflammation and ischemic cerebral injury by regulating anti-inflammatory molecules (IL-4 and IL-10) and pro-inflammatory cytokines (IL-6, TNF-α and IL-1β) (37). Despite the controversy on the microglia phenotypic classification, the majority of the current preclinical experiments have opted to explore the treatment strategy of microglial polarization with a broad concept of the M1 and M2 phenotypes (15). M2 microglia release anti-inflammatory mediators, whereas M1 microglia secrete pro-inflammatory cytokines to destroy adjacent neurons and oligodendrocytes. Some experimental results have indicated that in M2 microglia-derived extracellular vesicles, down-regulation of thioredoxin interacting protein mediates NLRP3 inflammasome expression via miR-135a-5p, which has been further verified to function as a novel therapeutic target for repressed neuronal autophagy and alleviate ischemic brain injury (29). Hu *et al* (15) demonstrated that adipose stem cell-derived extracellular vesicles regulated microglial polarization, and the possible underlying mechanism may be related to the inhibition of the expression of STAT1 and PTEN. Moreover, Li *et al* (19) first reported that dental pulp stem cell-derived exosomes exerted a neuroprotective effect against neuro-inflammation in mice with cerebral I/R by inhibiting the high mobility group box 1 (HMGB1)/Toll-like receptor (TLR)4/MyD88/NF-κB signaling pathway. Liu *et al* (31) innovatively found that exosomes from bone marrow-derived mesenchymal stem cells inhibited the inflammatory response and improved neurological function by activating TGR5 to affect cAMP and NF-κB signaling and reduce the production of inflammatory factors in the MCAO model. The pooled analyses demonstrated that anti-inflammation remains key to the treatment of ischemic injury, and targeting extracellular vesicles related to it is a promising therapeutic direction.

With new insight into the nature of extracellular vesicle-mediated intercellular communications, miRNAs have become the most extensively studied molecules in extracellular vesicles (38). miRNAs as single stranded non-coding RNAs, play crucial roles in mediating a range of biological functions and regulating post-transcriptional protein expression (8). To date, there are studies that suggest that miRNAs may be candidates for innovative gene therapy, playing multiple roles in promoting neurogenesis, angiogenesis and neuroplasticity. The delivery system for miRNAs has been developed to improve the biologic efficiency (39). In the studies included in the present meta-analysis, six related miRNAs were involved, namely miR-124 (25,28), miR-26a (22), miR-132 (27), miR-206 (32), miR-23a-3p (30), miR-135a-5p (29). Li *et al* (25) demonstrated that M2-derived extracellular vesicles reduced glial scar formation and inhibited astrocyte inflammation in mice by decreasing the expression of STAT3 and p-STAT3. Moreover, Song *et al* (28) reported that miR-124 in M2 microglia-derived exosomes promoted neuronal survival, and the mechanism involved may be related to miR-124 and its downstream target, ubiquitin specific peptidase 14 (USP14). These findings demonstrated that extracellular vesicles play a neuroprotective role by regulating miR-124 and decreasing the expression of its downstream target, STAT3/p-STAT3/USP14. In summary, these results illustrated that miRNAs exert a significant neuroprotective effect and promote neuro-recovery, thus providing more possibilities for the treatment of ischemic stroke.

To date, there is increasing evidence to indicate that extracellular vesicle therapy is the safer and more effective treatment strategy with which to combat cerebral ischemic injury. An increasing number of studies are being conducted to expand the possibilities of the therapeutic mechanisms and modes of extracellular vesicles. However, the experimental analysis and clinical application of extracellular vesicles are still faced with numerous challenges. First, the large-scale production, isolation and purification of extracellular vesicles continue to be urgent technical issues that need to be resolved in clinical applications. Second, the specific treatment plan of extracellular vesicle therapy and the measurement indices of efficacy evaluation still need to be more standardized. For example, in the studies included in the present meta-analysis, there was no uniform measurement standard for the evaluation of neurobehavioral function following I/R in mice. Finally, several limitations in the present meta-analysis itself should be mentioned, such as: i) The present study was limited to literature published in English before June 2023 and did not include unpublished articles; ii) the majority of included studies used healthy mice/rats to create I/R models. However, in clinical practice, a number of basic diseases are all high-risk factors for ischemic stroke, such as hypertension, diabetes and hyperlipidemia; and iii) the present study used WebPlotDigitizer (version 2.26, https://apps.auto-meris.io/wpd/index.zh_CN.html) software to extract part of the data from graphics, which may alter the original data to a certain extent and may thus have caused errors in the results.

In conclusion, extracellular vesicles can effectively improve nerve function and reduce infarct volume following ischemic stroke. Additionally, the present study identified that stem cell-derived extracellular vesicles can reduce the expression of IL-6, IL-1β and TNF-α and inhibit the neuroinflammatory response by inhibiting the activation of MAPK/cAMP/HMGB1/TLR4/MyD88/NF-κB signaling pathway. miRNA may can impact protein translational suppression and activation of numerous gene targets. In the current study, extracellular vesicles were found to promote neuronal survival and exert neuroprotective effects via six related miRNAs, namely miR-124, miR-26a, miR-132, miR-206, miR-23a-3p and miR-135a-5p; and targeting and upregulating/downregulating the expression of STAT1, PTEN, USP14 and TXNZP, the potential downstream targets of miRNAs. Although some factors, such as publication bias may

have influenced the heterogeneity of results, it is considered that the present meta-analysis provides an important basis for clinical trials on extracellular vesicles. At the same time, it also suggests that the experimental design should be optimized when conducting preclinical experiments in an aim to minimize bias.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YL contributed to conception and the design of the study. YuX and XH conducted the literature search and data extraction. JM and DZh assisted in the analysis and interpretation of the data. YuX performed the data analysis and wrote the draft. YL participated in writing and revising the manuscript. TD, LX and YaX inspected and proofed this systematic review and the final manuscript, YuX and YL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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