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Research Article

# Mesenchymal stem cell-derived extracellular vesicles therapy in traumatic central nervous system diseases: a systematic review and meta-analysis

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## Abstract

Although there are challenges in treating traumatic central nervous system diseases, mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) have recently proven to be a promising non-cellular therapy. We comprehensively evaluated the efficacy of mesenchymal stem cell-derived extracellular vesicles in traumatic central nervous system diseases in this meta-analysis based on preclinical studies. Our meta-analysis was registered at PROSPERO (CRD42022327904, May 24, 2022). To fully retrieve the most relevant articles, the following databases were thoroughly searched: PubMed, Web of Science, The Cochrane Library, and Ovid-Embase (up to April 1, 2022). The included studies were preclinical studies of mesenchymal stem cell-derived extracellular vesicles for traumatic central nervous system diseases. The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)'s risk of bias tool was used to examine the risk of publication bias in animal studies. After screening 2347 studies, 60 studies were included in this study. A meta-analysis was conducted for spinal cord injury ($n$ = 52) and traumatic brain injury ($n$ = 8). The results indicated that mesenchymal stem cell-derived extracellular vesicles treatment prominently promoted motor function recovery in spinal cord injury animals, including rat Basso, Beattie and Bresnahan locomotor rating scale scores (standardized mean difference [SMD]: 2.36, 95% confidence interval [CI]: 1.96–2.76, $P$ < 0.01, $I^2$ = 71%) and mouse Basso Mouse Scale scores (SMD = 2.31, 95% CI: 1.57–3.04, $P$ = 0.01, $I^2$ = 60%) compared with controls. Further, mesenchymal stem cell-derived extracellular vesicles treatment significantly promoted neurological recovery in traumatic brain injury animals, including the modified Neurological Severity Score (SMD = –4.48, 95% CI: –6.12 to –2.84, $P$ < 0.01, $I^2$ = 79%) and Foot Fault Test (SMD = –3.26, 95% CI: –4.09 to –2.42, $P$ = 0.28, $I^2$ = 21%) compared with controls. Subgroup analyses showed that characteristics may be related to the therapeutic effect of mesenchymal stem cell-derived extracellular vesicles. For Basso, Beattie and Bresnahan locomotor rating scale scores, the efficacy of allogeneic mesenchymal stem cell-derived extracellular vesicles was higher than that of xenogeneic mesenchymal stem cell-derived extracellular vesicles (allogeneic: SMD = 2.54, 95% CI: 2.05–3.02, $P$ = 0.0116, $I^2$ = 65.5%; xenogeneic: SMD: 1.78, 95%CI: 1.1–2.45, $P$ = 0.0116, $I^2$ = 74.6%). Mesenchymal stem cell-derived extracellular vesicles separated by ultrafiltration centrifugation combined with density gradient ultracentrifugation (SMD = 3.58, 95% CI: 2.62–4.53, $P$ < 0.0001, $I^2$ = 31%) may be more effective than other EV isolation methods. For mouse Basso Mouse Scale scores, placenta-derived mesenchymal stem cell-derived extracellular vesicles worked better than bone mesenchymal stem cell-derived extracellular vesicles (placenta: SMD = 5.25, 95% CI: 2.45–8.06, $P$ = 0.0421, $I^2$ = 0%; bone marrow: SMD = 1.82, 95% CI: 1.23–2.41, $P$ = 0.0421, $I^2$ = 0%). For modified Neurological Severity Score, bone marrow-derived MSC-EVs worked better than adipose-derived MSC-EVs (bone marrow: SMD = –4.86, 95% CI: –6.66 to –3.06, $P$ = 0.0306, $I^2$ = 81%; adipose: SMD = –2.37, 95% CI: –3.73 to –1.01, $P$ = 0.0306, $I^2$ = 0%). Intravenous administration (SMD = –5.47, 95% CI: –6.98 to –3.97, $P$ = 0.0002, $I^2$ = 53.3%) and dose of administration equal to 100 μg (SMD = –5.47, 95% CI: –6.98 to –3.97, $P$ < 0.0001, $I^2$ = 53.3%) showed better results than other administration routes and doses. The heterogeneity of studies was small, and sensitivity analysis also indicated stable results. Last, the methodological quality of all trials was mostly satisfactory. In conclusion, in the treatment of traumatic central nervous system diseases, mesenchymal stem cell-derived extracellular vesicles may play a crucial role in promoting motor function recovery.

**Key Words:** animals; central nervous system diseases; extracellular vesicles; mesenchymal stromal cell; meta-analysis; spinal cord injury; traumatic brain injury

## Introduction

The central nervous system includes the spinal cord and brain, and traumatic central nervous system diseases mainly refer to spinal cord injury (SCI) and traumatic brain injury (TBI), which are increasingly recognized as global health priorities (Maas et al., 2008; Ahuja et al., 2017a). TBI is often characterized by mental decline, hearing and vision loss, hemiplegia, and even coma and other related symptoms (Andriessen et al., 2011). SCI can cause paraplegia below the innervated plane (Ahuja et al., 2017b). These injuries not only lead to reduced quality of life for the affected individuals and their families but also become a burden to society due to productivity losses and high health care costs (Young et al., 2019). Current treatments for SCI include early surgical decompression (Ramakonar and Fehlings, 2021), glucocorticoid pulse therapy (Bracken et al., 1997), and neurotrophic drug therapy (Hurlbert et al., 2013). For TBI, the treatments include rehabilitation training and pharmacological support (Nelson et al., 2019). However, none of these treatments can improve the patient's neurological recovery to a great extent (Maas et al., 1999). Therefore, new therapeutic approaches are urgently needed to prevent or slow down the progression of secondary injury in traumatic central nervous system diseases.

Mesenchymal stem cells (MSCs) have been widely studied as a therapeutic option for traumatic central nervous system diseases (Tetzlaff et al., 2011; Harrop et al., 2012; Hachem et al., 2017; Wen et al., 2022; Zhang et al., 2022). However, when cell transplantation is applied in clinical studies, tumorigenicity and immune rejection become obstacles to its clinical application (Liu et al., 2021b). It has been shown that the significant efficacy of MSCs is attributable to the extracellular vesicles (EVs) they secrete

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(Pinho et al., 2020; Yari et al., 2022). Extracellular vesicles are intercellular communication tools, which are divided into several subtypes: apoptotic bodies, ectosomes or shedding microvesicles, and exosomes (Colombo et al., 2014; Kalra et al., 2016). Exosomes are small EVs originating from the endosomes and measuring 40–150 nm. Ectosomes or shedding microvesicles are large EVs with a diameter between 100 and 1000 nm, and are secreted by the plasma membrane. Apoptotic cells release heterogeneous EVs, called apoptotic bodies, with a diameter of 50–5000 nm. These vesicles partially overlap in size. Therefore, there are great challenges in the separation of pure EV subtypes (Lotvall et al., 2014). A number of comprehensive reviews regarding the different sources, contents, and functions of these types of vesicles are available (Théry et al., 2018; van Niel et al., 2018; Jeppesen et al., 2019). EVs contain various RNAs and proteins that play an anti-inflammatory, anti-apoptotic, and neuroprotective roles in traumatic central nervous system disease therapy (Li et al., 2020b; Yang et al., 2022). They can not only replace the damaged cells but also compensate for the disadvantages of cell therapy, such as low immunogenicity and the role of crossing the blood-brain barrier (Théry et al., 2002).

Although EVs have received much attention, there are still a number of issues that need to be addressed regarding this cell-free therapy. A conference on EVs has presented existing relevant questions and solutions (Théry et al., 2018). However, there is no consensus on the method of EV isolation, the source of cells, EV subtypes, and the maximum benefit from the dosing regimen. An omnidirectional and systematic grasp of these experimental approaches and the efficacy of MSC-EVs for traumatic central nervous system diseases are needed for the preclinical studies to clinical translation. Hence, we performed a systematic review and meta-analysis of recent animal model studies that used MSC-EVs for traumatic central nervous system diseases. We also performed subgroup analyses based on MSC origin, EV isolation methods, subtypes, and dosage regimen. Last, we performed a bias risk assessment and a sensitivity analysis to assess the stability of the results.

## Methods

The protocol for this study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) guidelines (Page et al., 2021). The study protocol was registered in the PROSPERO database (registeration No. CRD42022327904) on May 24, 2022.

### Search strategy

To fully retrieve most of the articles, the following databases were retrieved: PubMed, Web of Science, The Cochrane Library, and Ovid-Embase (up to April 1, 2022). References were also reviewed for relevance and manual studies of included articles. Only the articles were limited to English-language publications were considered. Comprehensive information on the search strategy is provided in **Additional file 1**.

### Data extraction

The types of literature were screened by two investigators (the first and second authors ZY and ZY) following the inclusion and exclusion criteria. Any differences were settled through consultation; otherwise a third party (the corresponding author CC) was consulted.

### Inclusion and exclusion criteria

The eligibility criteria were strictly formulated in accordance with Population Intervention Comparison Outcome Study design (PICOS) principles.

**Study subjects:** We included all animal studies of traumatic central nervous system diseases and excluded other invertebrates and *in vitro* studies.

**Interventions:** We included all studies of MSC-EVs for traumatic central nervous system diseases and excluded those of other cell-derived EVs.

**Comparisons:** All comparison groups were considered, including those treated with phosphate buffer saline, untreated groups, and negative controls.

**Outcomes:** The outcome measures were the Foot Fault Test and the modified Neurological Severity Score (mNSS), which could be used to evaluate neurological function in animals with TBI. Further, the Basso, Beattie and Bresnahan (BBB) locomotor rating scale and Basso Mouse Scale (BMS) scores were used to assess hindlimb motor function in rats and mice with SCI, respectively.

**Studies:** We included controlled intervention studies (randomized or non-randomized), whereas reviews, comments, letters, and unpublished studies were excluded.

### Data collection and bias risk evaluation

The data were independently collected and cross-checked by two professional researchers (the authors ZY and ZL) from the screened studies. Any disagreement was resolved through consultation with the third party (the corresponding author CC). The data extracted: (a) study characteristics: author, year, country, the sample size of each group, animal, sex, weight, TBI and SCI models, MSC source, immunocompatibility, EV isolation and size/morphology analysis, EV positive markers, EV negative markers, dosage regimen (time, dosage, number of doses, and route); and (b) outcomes: mNSS, Foot Fault Test, BBB, and BMS. The quality of the studies included by the two researchers was analyzed using the SYRCLE's risk of bias tool (https://www.biomedcentral.com/1471-2288/14/43) (Hooijmans et al., 2014) for animal studies, including attrition bias, performance bias, reporting bias, selection bias, detection bias, and other considerations from a list of 10 entries.

### Outcome measurements

Neurological assessment in animals with TBI included the mNSS and the Foot Fault Test. BBB and BMS were used as outcome measures to determine hindlimb motor function in rats and mice with SCI, respectively.

### Statistical analysis

Each outcome was analyzed with a 95% confidence interval (CI) for continuous outcomes using the standardized mean difference (SMD). The $I^2$ test was used to evaluate statistical heterogeneity. This test exhibits remarkable heterogeneity when $I^2$ values exceed 50%, and in these cases, a random-effects model was used; otherwise, a fixed effects model was used. The results were summarized graphically using forest plots. We assessed the stability of the results by performing sensitivity analysis using the exclusion method. Meta-analysis was performed with the R software (version 4.1.3; Boston, MA, USA). A *P*-value of 0.05 was set to determine statistical significance. The funnel plots and Egger's regression test were used to evaluate publication bias. Planned subgroup analyses included animal-based characteristics (e.g., sex and model); intervention characteristics (e.g., tissue source of MSCs, EV subtype, EV isolation methods, and immunocompatibility); and dosing regimen (time, dose, and route).

## Results

### Literature retrieval

A total of 2347 studies were initially identified after a systematic search of PubMed, Web of Science, The Cochrane Library, and Ovid-Embase databases. Subsequently, 172 replicate studies were excluded. A total of 2098 studies were deleted after screening the title and abstract, and the reasons for exclusion are presented in **Figure 1**. Then, we carefully searched the full text of the remaining 77 studies for evaluation. Subsequently, 17 studies were excluded for various reasons (**Figure 1**). Finally, 60 studies were included in this study.

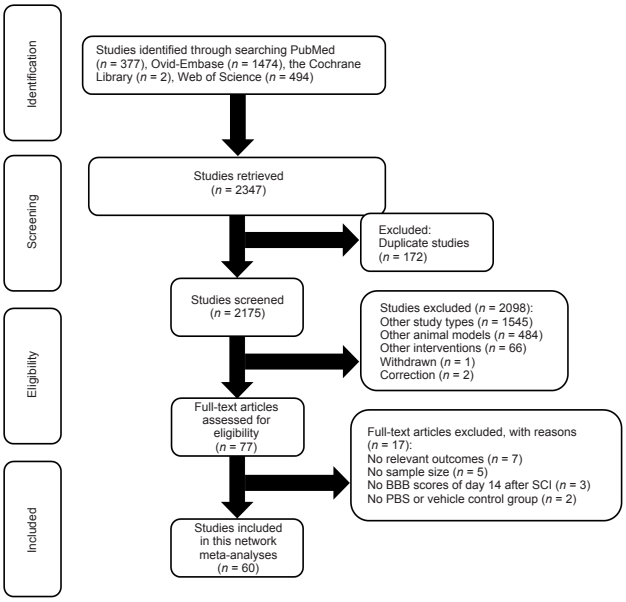


**Figure 1** | **Flowchart of article selection process.**

### Study characteristics

The characteristics of the 60 included studies (Zhang et al., 2015, 2017, 2020a, b, 2021a, b, c; Huang et al., 2017, 2020a, b, 2021a, b, 2022; Kim et al., 2018; Li et al., 2018, 2019, 2020a, 2021; Ruppert et al., 2018; Sun et al., 2018; Wang et al., 2018, 2021a, b; Guo et al., 2019; Kang et al., 2019; Liu et al., 2019, 2020, 2021a; Lu et al., 2019; Ni et al., 2019; Yang et al., 2019; Yu et al., 2019; Zhao et al., 2019; Zhou et al., 2019, 2021, 2022; Chen et al., 2020, 2021; Gu et al., 2020; Lee et al., 2020; Xu et al., 2020; Chang et al., 2021; Cheng et al., 2021; Fan et al., 2021; Jia et al., 2021a, b, c; Jiang and Zhang, 2021; Luo et al., 2021; Mu et al., 2021; Nakazaki et al., 2021; Nie and Jiang, 2021; Romanelli et al., 2021; Sheng et al., 2021; Xiao et al., 2021; Xin et al., 2021; Zhai et al., 2021; Han et al., 2022; Kang and Guo, 2022; Liang et al., 2022) are summarized in **Additional Table 1**. The majority of the studies were performed in China (*n* = 52), with five studies in the United States, two studies in Korea, and one study in Australia. The sample size in each group ranged from 5 to 24. Most studies used rat models (*n* = 50), with only 10 studies using mouse models. The majority were male animals (*n* = 36), with 20 studies using female animals. However, four studies did not describe the sex of the animals. Rats weighed 80–300 g, and mice weighed 17–35 g; the age range was between 2 and 14 weeks. All TBI (*n* = 8) used the CCI compression models, and SCI (*n* = 52) models include contusion, compression, hemisection, and transection. The majority of the studies used MSCs derived from the bone

marrow (*n* = 42), a portion from the placenta (*n* = 10), and a small portion from fat (n = 5) and umbilical cord (*n* = 3). The origin of these stromal cells was both allogeneic (n = 34) and xenogeneic (*n* = 23). However, some studies did not provide this information (*n* = 3). EVs were isolated by ultracentrifugation (*n* = 32), isolation kit (*n* = 13), density gradient ultracentrifugation (*n* = 2) continuous extrusion, density gradient ultracentrifugation and magnetic sorting (*n* = 1), continuous filtration (*n* = 1), ultrafiltration centrifugation combined with ultracentrifugation (*n* = 4), ultrafiltration centrifugation combined with density gradient ultracentrifugation (*n* = 4), tangential flow filtration and ultracentrifugation (*n* = 1), ultracentrifugation, ultrafiltration and molecular exclusion chromatography (*n* = 1), polyethylene glycol and ultracentrifugation (*n* = 1). Fortunately, most studies (*n* = 56) took two or three approaches to EV identification according to the mid-September 2018 guidelines (Théry et al., 2018). However, there were four studies that took only one approach. Importantly, negative markers were not used to demonstrate specific isolation of EVs in many studies (n = 38), and only some studies (*n* = 22) reported negative markers. MSC-EVs were administered intravenously (*n* = 49), intrathecally (*n* = 10), intracerebroventricularly (*n* = 1), retroorbitally (*n* = 1), and intranasally (*n* = 1). Most studies (*n* = 41) delayed dosing after injury, and some studies (*n* = 18) dosed immediately after the injury. However, one study did not describe the time of dosing. Most studies (*n* = 23) used doses of MSC-EVs ≥ 100 μg protein, with 19 studies administering doses equal to 100 μg and 11 studies administering doses ≤ 100 μg. However, three studies did not use protein quantification, but EV particle number quantification, and four studies did not describe the dose. There were 41 studies with single dosing and 16 studies with multiple dosing. Three studies did not describe the number of doses.

**Methodological quality and risk of bias**

The methodological quality assessment charts and summaries of all studies included in this meta-analysis are shown in **Figure 2**. For the overall risk of bias assessment, of all studies included, 21 (35%) were high risk, 10 (17%) were low risk, and 29 (48%) showed unclear risk. In addition, half of the randomly selected outcome assessments for detection bias of the included studies were low risk and half were ambiguous. Sequence generation risk of selection bias and detection bias blinding were low for most included studies. However, most of the included studies showed unclear risks in many items such as baseline characteristics of selection bias, selection bias allocation concealment, performance bias, incomplete outcome data for attrition bias, and selective outcomes for reporting bias. In conclusion, the methodological quality of all trials was mostly satisfactory.

**Effect of MSC-EVs on motor function recovery after SCI**

The BBB score of the rats: a total of 43 included articles (Huang et al., 2017, 2020a, b, 2021a, b, , 2022; Li et al., 2018, 2019, 2020a; Ruppert et al., 2018; Wang et al., 2018, 2021a, b; Guo et al., 2019; Kang et al., 2019; Liu et al., 2019; Lu et al., 2019; Yu et al., 2019; Zhao et al., 2019; Zhou et al., 2019, 2021, 2022; Gu et al., 2020; Chang et al., 2021; Chen et al., 2021; Cheng et al., 2021; Fan et al., 2021; Jia et al., 2021a, b, c; Jiang and Zhang, 2021; Luo et al., 2021; Mu et al., 2021; Nakazaki et al., 2021; Nie and Jiang, 2021; Romanelli et al., 2021; Xiao et al., 2021; Xin et al., 2021; Zhang et al., 2021a, b; Han et al., 2022; Kang and Guo, 2022; Liang et al., 2022) were analyzed. The results indicated that MSC-EVs treatment significantly promoted motor function recovery in rats (SMD = 2.36, 95% CI: 1.96–2.76, $P < 0.01$, $I^2 = 71\%$; **Figure 3**). The results of subgroup analyses demonstrated that allogeneic MSC-EVs were more beneficial to motor function recovery than xenogeneic administration (allogeneic: SMD = 2.54, 95% CI: 2.05–3.02, $I^2 = 65.5\%$; xenogeneic: SMD = 1.78, 95% CI: 1.1–2.45, $I^2 = 74.6\%$, $P = 0.0116$). The EV isolation method (SMD = 3.58, 95% CI: 2.62–4.53, $P < 0.0001$, $I^2 = 31\%$) may be related to higher EV efficacy, as ultrafiltration centrifugation combined with density gradient ultrafiltration showed better results than other EV isolation methods (**Figure 4**). However, there were no differences in efficacy between the tissue source of MSC, EV subtype, route of administration, time administered, dose administered, animal's sex, and SCI model (**Figure 4**). Although the study showed heterogeneity, sensitivity analysis indicated that the results were stable (**Figure 1** and **Additional Figure 1**).

BMS score of the mice: nine included articles (Kim et al., 2018; Sun et al., 2018; Lee et al., 2020; Liu et al., 2020, 2021a; Zhang et al., 2020a; Li et al., 2021; Sheng et al., 2021; Zhai et al., 2021) were analyzed. The results indicated that MSC-EVs treatment significantly promoted motor function recovery in mice (SMD = 2.31, 95%CI: 1.57–3.04, $I^2 = 60\%$, $P = 0.01$) (**Figure 5**). Subgroup analysis demonstrated that placenta-derived MSCs had stronger motor function recovery than bone marrow-derived MSC (placenta: SMD = 5.25, 95% CI: 2.45–8.06, $I^2 = 0\%$; bone marrow: SMD = 1.82, 95% CI: 1.23–2.41, $I^2 = 0\%$; $P = 0.0421$), but only one study involved placenta-derived MSCs. The EV isolation method (SMD = 6.79, 95% CI: 3.97–9.67, $I^2 = 0\%$, $P = 0.0099$) may be related to higher EV efficacy, as ultrafiltration centrifugation combined with ultrafiltration showed better results than other EV isolation methods (**Figure 6**). Finally, the efficacy of MSC-EVs in the spinal cord contusion model was better than the compression model (compression: SMD = 1.41, 95% CI: 0.66–2.17, $I^2 = 0\%$; contusion: SMD = 2.72, 95% CI: 1.76–3.69, $I^2 = 63.2\%$; $P$=0.0365). However, there were no differences in efficacy between the immunocompatibility of MSCs, EV subtype, route of administration, treatment time, dose administered, and animal's sex (**Figure 6**). Despite heterogeneity between studies, sensitivity analysis indicated that the results were stable (**Figure 2** and **Additional Figure 2**).

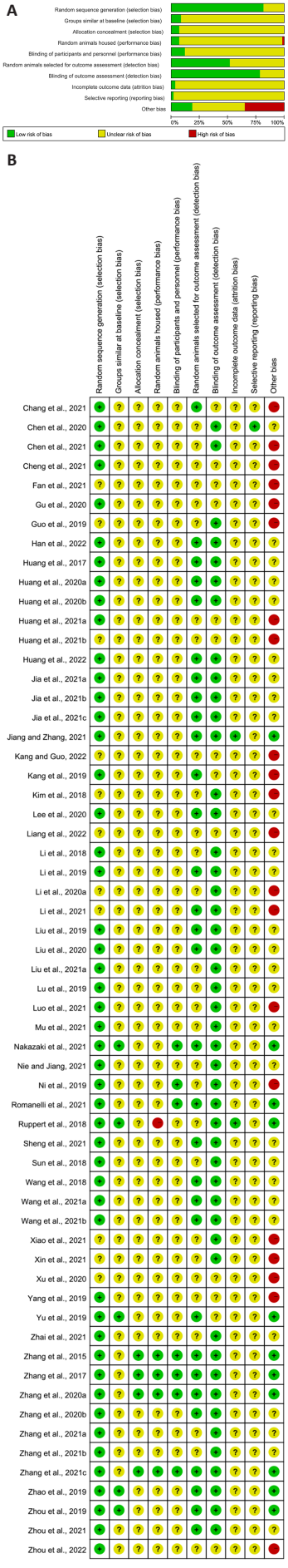


**Figure 2** | **Risk of bias with the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool.** (A) Risk of bias graph. (B) Risk of bias summary.

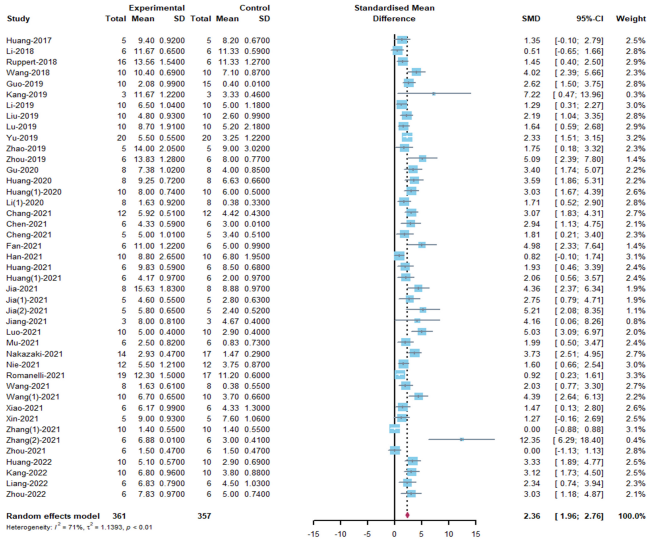


**Figure 3** | **Meta-analysis of Basso, Beattie and Bresnahan locomotor rating scale scores.**
References Wang 2021 and Wang (1) 2021 correspond to Wang et al., 2021a and Wang et al., 2021b in the reference list, respectively. References Zhang (1) 2021 and Zhang (2) 2021 correspond to Zhang et al., 2021a and Zhang et al., 2021b in the reference list.

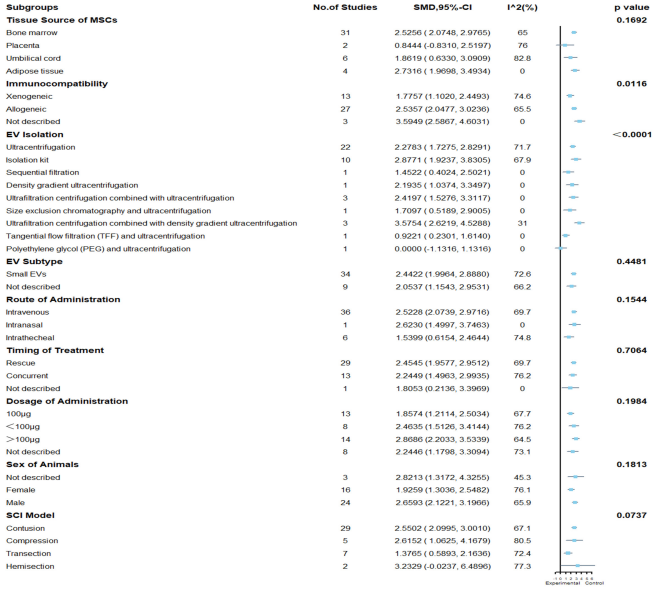


**Figure 4** | **Subgroup analysis of Basso, Beattie and Bresnahan locomotor rating scale scores.**
EV: Extracellular vesicle.

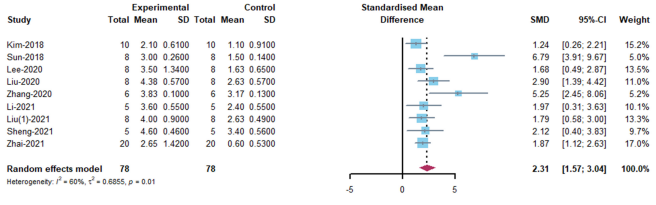


**Figure 5** | **Meta-analysis of the Basso Mouse Scale scores.**
The reference Liu (1) 2021 correspond to Liu et al., 2021a in the reference list.

### Effect of MSC-EVs on neurological recovery after TBI

**mNSS:** eight included articles (Zhang et al., 2015, 2017, 2020a, 2021c; Ni et al., 2019; Yang et al., 2019; Chen et al., 2020; Xu et al., 2020) were analyzed. The results indicated that MSC-EVs treatment significantly promoted neurological recovery in rats (SMD = –4.48, 95% CI: –6.12 to –2.84, $I^2$ = 79%, $P$ < 0.01; **Figure 7**). The results of subgroup analyses showed that bone marrow-derived MSCs showed a stronger recovery of neurological function than adipose-derived MSCs (bone marrow: SMD = –4.86, 95% CI: –6.66 to –3.06, $I^2$ = 81%; adipose: SMD = –2.37, 95% CI: –3.73 to –1.01, $I^2$ = 0%; $P$ = 0.0306). Administration route (SMD = –5.47, 95% CI: –6.98 to –3.97, $I^2$ = 53.3%, $P$ = 0.0002) and dose (SMD = –5.47, 95% CI: –6.98 to –3.97, $I^2$ = 53.3%, $P$ < 0.0001) may be related to higher EV efficacy, as intravenous administration and dose of administration equal to 100 μg showed better results than other administration routes and doses (**Figure 8**). However, there were no differences in efficacy among the immunocompatibility of MSCs, EV isolation methods, and EV subtypes (**Figure 8**). Despite heterogeneity between studies, sensitivity analysis indicated that the results were stable (**Figure 3** and **Additional Figure 3**).

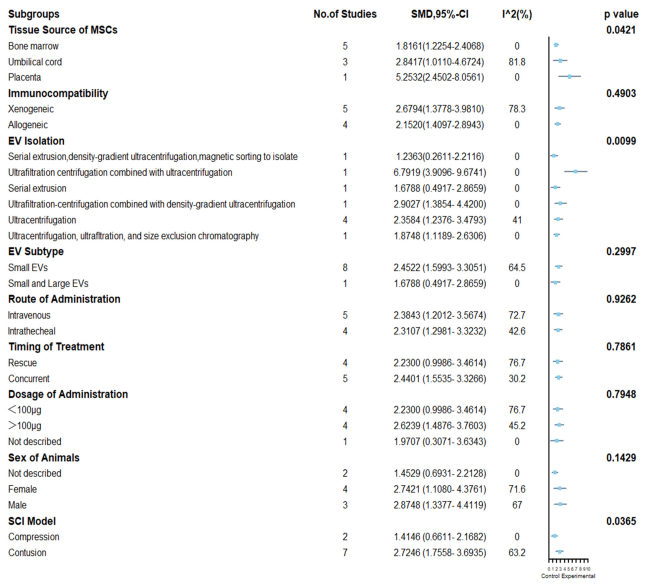


**Figure 6** | **Subgroup analysis of Basso Mouse Scale scores.**
EV: Extracellular vesicle; MSCs: mesenchymal stem cells.

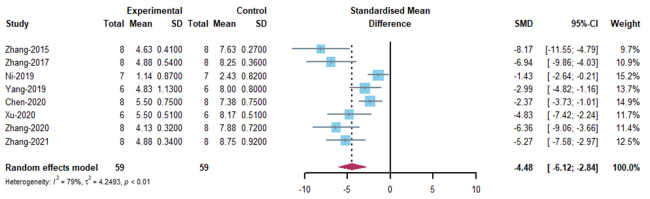


**Figure 7** | **Meta-analysis of modified Neurological Severity Scores.**
The references Zhang-2020 and Zhang-2021 correspond to Zhang et al., 2020a and Zhang et al., 2021c in the reference list, respectively.

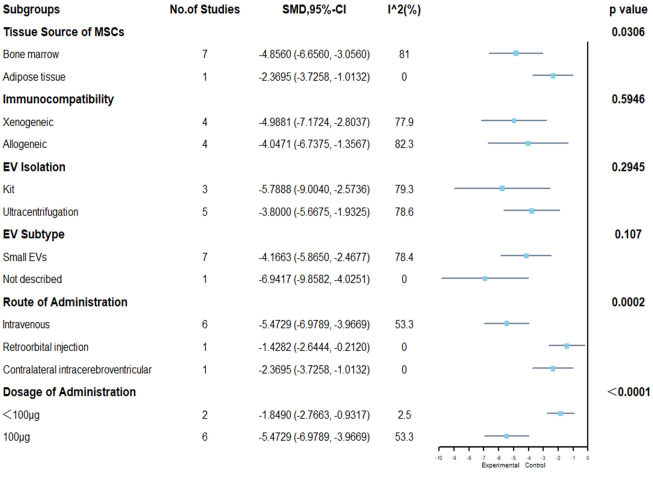


**Figure 8** | **Subgroup analysis of modified Neurological Severity Scores.**
EV: Extracellular vesicle; MSCs: mesenchymal stem cells.

**Foot Fault Test:** five included articles (Zhang et al., 2015, 2017, 2020b, c; Chen et al., 2020) were analyzed. The results showed that MSC-EVs treatment significantly promoted neurological recovery in rats (SMD = −3.26, 95% CI: −4.09 to −2.42, $I^2$ = 21%, $P$ = 0.28; **Figure 9**). The results of subgroup analyses demonstrated that no efficacy differences were observed between the tissue origin, immunocompatibility, EV isolation method, EV subtype, route of administration, and dose of administration of MSCs (**Figure 10**). The heterogeneity of the studies was small, and sensitivity analysis also indicated stable results (**Figure 4** and **Additional Figure 4**).

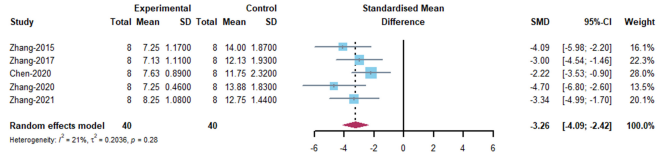


**Figure 9** | **Meta-analysis of Foot Fault Test results.**
The references Zhang-2020 and Zhang-2021 correspond to Zhang et al., 2020a and Zhang et al., 2021c in the reference list, respectively.

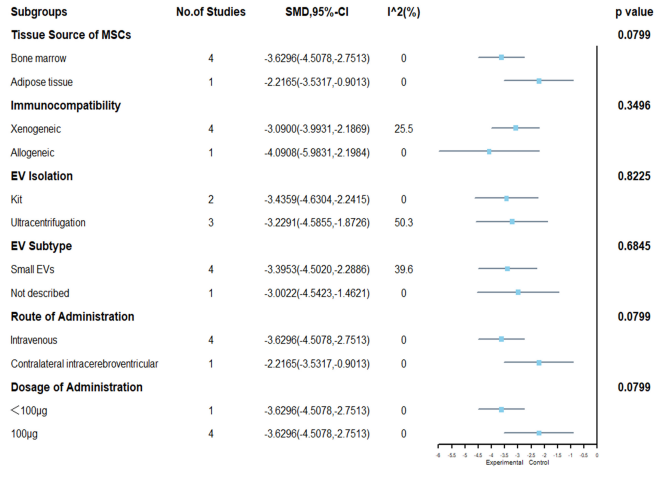


**Figure 10** | **Subgroup analysis of Foot Fault Test results.**
EV: Extracellular vesicle; MSCs: mesenchymal stem cells.

### Publication bias

We used Funnel plots and Egger's regression test to evaluate publication bias (**Figure 11**). There was a significant publication bias in the funnel plot for visual inspection of rat BBB scores. Egger regression confirmed this result and also showed evidence of publication bias (Egger's test: $t$ = 6.27, df = 41, $P$ < 0.0001). The absence of 16 articles on the left (unfilled circles) could have been predicted by pruning and filling analysis. Because the number of articles for the other outcome measures was ≤ 10, no publication bias assessment was performed for the other outcome measures.

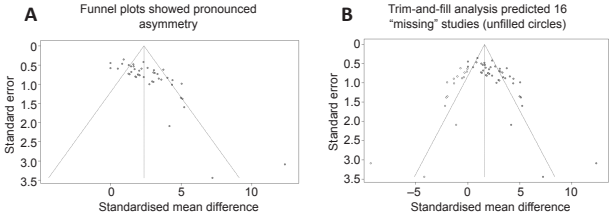


**Figure 11** | **Assessment of publication bias in Basso, Beattie and Bresnahan locomotor rating scale scores.**
(A) Funnel plots showed pronounced asymmetry. (B) Trim-and-fill analysis predicted 16 "missing" studies (unfilled circles). The dotted lines represent 95% confidence intervals.

## Discussion

Our systematic review comprehensively synthesizes the preclinical study design, methods, therapeutic effect, and preclinical reports of studies of MSC-EVs for traumatic central nervous system diseases. The results showed that MSC-EVs administration obviously promoted outcome measures of traumatic central nervous system diseases, as assessed by the BBB, BMS, mNSS, and Foot Fault tests. These findings show the therapeutic effect of MSC-EVs for traumatic central nervous system diseases by significantly improving motor recovery in animals with SCI and neurological recovery in animals with TBI.

Our meta-analysis systematically evaluated the efficacy of MSC-EVs for traumatic central nervous system diseases from various perspectives of experimental approaches. Given that the development of MSC-EVs therapies involves many variables, we performed a meta-analysis to evaluate relevant factors that may enhance the efficacy of EVs. In the study of SCI in rats, allogeneic administration of MSC-EVs may be more helpful for motor function recovery than xenogeneic administration, which may be because allogeneic administration of MSC-EVs has low immunogenicity and low immune rejection, thereby increasing their survival (van Balkom et al., 2019). In addition, EV obtained using ultrafiltration centrifugation combined with density gradient ultrafiltration may be associated with higher efficacy. Because the higher the purity of EV obtained by separation, the clearer the function, while a single separation method will likely produce many pollutants, which may have a negative impact on the function. A study has shown that the combination of 3D-cultured MSC and tangential flow filtration can obtain higher yield and purity of MSC-EVs (Haraszti et al., 2018). This shows that the combined separation method may be superior to the single separation method (Tieu et al., 2020), which was consistent with minimal information on extracellular vesicle studies (mid-September 2018).

In studies of SCI in mice, while only one study showed stronger motor recovery using placenta-derived MSC-EVs, it is well-known that the placenta is less likely to produce immune rejection. Because it is designated as biohazardous waste, it can be used as a non-invasive and rich source of stem cells (Hua et al., 2013). Therefore, easy availability of the placenta shows its ethical advantages when considering clinical translation. As in studies of SCI in rats, EVs obtained using ultrafiltration centrifugation combined with ultrafiltration may show better results than other EV isolation methods. Finally, MSC-EVs may be more effective in treating the SCI contusion model than the SCI compression model, possibly because contusion is the oldest and most commonly used method for SCI models (Sharif-Alhoseini et al., 2017), and the stability of the model can be controlled using parameterization to make the model more reproducible (Pearse et al., 2005). MSC-EVs have been shown to well inhibit the inflammatory response at the time of the cascade inflammatory response early in SCI, which may be related to its better efficacy in contusion models. The results in studies of TBI have shown that bone marrow-derived MSC-EVs showed stronger neurological recovery than other origins, which may be related to only one study of fat origin. However, it is difficult to obtain bone marrow-derived MSCs when it is used for clinical transformation (Kern et al., 2006). Therefore, it is important to choose the source of MSC-EVs. In addition, intravenous administration and the 100 μg dose showed better results, which shows that intravenous administration is safer, more controllable, and produces fewer side effects than other modalities. It also suggests that 100 μg may be the optimal dose at which EVs work and that a higher dosage is perhaps a burden for animals and is also more likely to produce toxicity or side effects. Some studies have shown that a single dose of EVs administered early can have a significant effect (Williams et al., 2020; Bambakidis et al., 2022). However, other studies have demonstrated that multiple doses of the same EVs are more effective than single doses (Nakazaki et al., 2021). Therefore, the dose and frequency of MSC-EVS administration still need to be further studied. Importantly, this study analyzed the quality of the included studies using the internationally accepted symbol animal study risk of bias tool. The methodological quality of all included studies was also satisfactory.

This meta-analysis has some limitations. First, the body weight of the rat was not considered. Second, in addition to studies of BBB scores in rats, the number of studies and sample size of other outcome indicators are very small, which may cause risk of bias. Third, these functional scores, using BBB, BMS, mNSS, and Foot Fault Tests as indicators for efficacy evaluation, are not comprehensive enough. It is remarkably subjective. Fourth, the funnel plot showed significant publication bias, which may be related to the fact that the included studies were preclinical studies. Last, most of the included studies showed unclear risks in many items. Only 17% of the studies had a low risk of bias, mainly because the studies we included and analyzed were preclinical studies (Begley and Ioannidis, 2015).

Preclinical studies of MSC-EVs are essential for their subsequent clinical application. A considerable number of articles have assessed the consequence of MSC-EVs in traumatic central nervous system diseases. However, no trial has directly compared the efficacy of different tissue- or cell-derived MSC-EVs in traumatic central nervous system diseases. This finding provides directions for future research. In addition, there is also no optimal parameter for the dose, route, and method of administration of MSC-EVs. Therefore, the optimal administration parameters of EVs should be the focus of future research. Most of the included studies demonstrated the effectiveness of EVs for traumatic central nervous system diseases. However, there is only one study on its safety (Huang et al., 2021a), which shows that MSC-EVs do not cause damage to the liver and lungs. Therefore, attention should be paid to the safety aspect of using MSC-EVs.

## Conclusion

In the treatment of traumatic central nervous system diseases, MSC-EVs may play a crucial role in promoting motor function recovery. However, through comprehensive analysis of the experimental methods and EV parameters of

the included studies, we believe that there is still some heterogeneity among the various studies that affect the results of the current study. Therefore, further standardization of preclinical trials is needed to promote clinical translation.

**Author contributions:** *ZY first proposed the idea and designed the outline of the article. ZL and JR were both responsible for all data extraction and analysis. The first version was prepared by ZY. ZL and FL participated in the article revision. YL and XX were in charge of chart making. The final version was revised by CW and CC. All authors reviewed and approved the final manuscript.*

**Conflicts of interest:** *None of the authors declare a conflict of interest.*

**Data availability statement:** *All relevant data are within the paper and its Additional files.*



**Editor's evaluation:** *Mesenchymal stem cell (MSC) transplantation has been widely studied as a treatment for central nervous system injury and diseases for decades, and MSC-derived extracellular vesicles (MSC-EVs), a cell-free therapy is drawing more attention in fields recently. In this manuscript, the authors screened all the published papers on MSC-EVs for the therapy in traumatic central nervous system diseases. It's very interesting that 60 studies were included according to the author's standard. Furthermore, they concluded that MSC-EVs treatment significantly promoted motor function recovery and neurological recovery in spinal cord injury and traumatic brain injury. Moreover, the authors concluded that placenta-derived MSC-EVs were more effective than bone marrow-derived MSC-EVs, intravenous administration and dose of administration equal to 100 μg had better effect. Therefore, MSC-EVs may play a significant role in improving motor function recovery in the treatment of traumatic central nervous system diseases. The novelty of the current study, which conducted a systematic review and meta-analysis to compare the benefits from the method of EVs isolation, the source of cells, EVs subtypes and dosing regimen, is to provide the foundation for future standardization of preclinical trials and clinical translation.*

**Additional files:**

***Additional file 1:*** *Search query for databases.*

***Additional Table 1:*** *Characteristics of the included studies.*

***Additional Figure 1:*** *Sensitivity analysis of Basso, Beattie and Bresnahan locomotor rating scale scores.*

***Additional Figure 2:*** *Sensitivity analysis of mouse Basso Mouse Scale scores.*

***Additional Figure 3:*** *Sensitivity analysis of modified Neurological Severity Score.*

***Additional Figure 4:*** *Sensitivity analysis of Foot Fault Test results.*

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