Brain Research Bulletin 230 (2025) 111481

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Review

Stem cell derived extracellular vesicles therapy for perinatal brain injury: A systematic review & meta-analysis of preclinical studies and a potential path to clinic

Xiaolin Guo [a,b,1], Tingting Peng [a,1], Mengru Zhong [a], Simian Cai [a], Lu He [a,*], Kaishou Xu [a,*]

[a] Department of Rehabilitation, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510120, China
[b] School of Exercise and Health, Shanghai University of Sport, Shanghai 200438, China

ARTICLE INFO

Keywords:
Perinatal brain injury
Stem cells
Extracellular vesicles
Cognition
Motor function
Preclinical studies

ABSTRACT

Background: Perinatal brain injury (PBI) is a significant cause of neonatal death and childhood disability. Current treatments for PBI are limited and ineffective. Stem cell derived extracellular vesicles (SC-EVs) have shown promising therapeutic potential in addressing PBI. We aimed to assess the effectiveness and potential mechanisms of SC-EVs therapy on behavioral and pathological outcomes in animal models of PBI.

Methods: We searched six databases (MEDLINE, Embase, Scopus, PubMed, ProQuest, and Web of Science) for articles on the therapeutic effects of SC-EVs in animal models of PBI. We extracted neurobehavioral and pathological results related to brain injury and used a random-effects model to calculate the standardized mean difference and confidence interval.

Results: Twenty-five articles met the inclusion criteria. Treatment with SC-EVs improved cerebral infarct size and tissue edema, as well as the recovery of cognition and motor function. The mechanism of action may be related to the inhibition of apoptosis, microglia activation, astrogliosis, and pro-inflammatory factor release, further promoting neuronal protection, remyelination, and angiogenesis. Study quality assessment found no studies to be at high risk, and there was significant heterogeneity among studies. Sensitivity analysis and subgroup analysis did not identify the source of heterogeneity.

Conclusion: SC-EVs might improve cognitive and motor functions, as well as brain microstructure, by exerting anti-apoptotic and anti-neuroinflammatory effects. This provides a theoretical basis for using cell-free therapies to prevent and treat PBI and supports the translation of SC-EVs from preclinical models to human applications.

1. Introduction

Perinatal brain injury (PBI) is damage to the developing brain during pregnancy or around the time of birth. PBI is a major cause of neonatal mortality and childhood disability, potentially leading to cerebral palsy, epilepsy, and other permanent neurological disorders (Leavy and Jimenez Mateos, 2020; Novak et al., 2018). Currently, available treatments for PBI are limited. Prenatal maternal magnesium sulfate infusion and hypothermia treatment are the most widely known interventions to prevent brain injury during the acute phase of PBI (Alpay Savasan et al., 2021). While maternal magnesium sulfate-based treatment before early preterm birth could decrease the risk of cerebral palsy in survivors, it is associated with maternal side effects, and high doses have been linked to negative outcomes for the fetus in terms of motor and personal-social function (Shaw and Yager, 2019; Yates et al., 2021). Therapeutic hypothermia is a proven treatment for ameliorating neurological dysfunction induced by PBI (Wassink et al., 2019). When initiated within 6 h of birth and maintained for 72 h of cooling (whole body or selective head), it significantly reduces death and improves neurodevelopmental outcomes in survivors (Proietti et al., 2024; Sabir et al., 2021). However, the International Liaison Committee of Resuscitation only recommends its application in term or near-term neonates (≥36 weeks) with moderate or severe encephalopathy, with initiation within the first 6 postnatal hours (Laptook et al., 2017; Perlman, 2010). Therefore, this prompts a continuous search for new long-term therapeutic options after the acute phase of PBI.

Stem cells show great therapeutic potential in a wide time window

* Corresponding authors.
E-mail addresses: kittyhelu@126.com (L. He), xksyi@126.com (K. Xu).
[1] Xiaolin Guo and Tingting Peng are co-first authors.

https://doi.org/10.1016/j.brainresbull.2025.111481
Received 12 February 2025; Received in revised form 8 June 2025; Accepted 23 July 2025
Available online 27 July 2025

Abbreviations

CD31	platelet endothelial cell adhesion molecule 1
CI	confidence interval
GFAP	glial fibrillary acidic protein
HI	hypoxic-ischemic brain injury
Iba-1	ionized calcium binding adaptor molecule 1
IL-1β	interleukin-1β
IL-6	interleukin-6
MBP	myelin basic protein
miR	microRNA
MSCs	mesenchymal stem cells
NeuN	neuronal nuclei antigen
NTA	nanoparticle tracking analysis
PBI	perinatal brain injury
PE	preeclampsia
SC-EVs	stem cell-derived extracellular vesicles
SD	standard deviation
SE	standard error
SMD	standardized mean difference
TEM	transmission electron microscopy
tMCAO	transient middle cerebral artery occlusion
TNF-α	tumor necrosis factor alpha
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
WB	western blot

after PBI (Purcell et al., 2023; Titomanlio et al., 2011). Recent studies have shown that the therapeutic effects of stem cells are more dependent on their paracrine pathway than on the replacement of damaged cells (Jafarinia et al., 2020; Katsha et al., 2011). Stem cell derived extracellular vesicles (SC-EVs), a lipid bilayer closed structure between 30 and 1000 nm, are the primary mediator of their paracrine secretion (Sisa et al., 2019; Théry et al., 2018). Recent studies have shown that therapeutic factors secreted by SC-EVs (including anti-inflammatory mediators, cytokines, and growth factors, as well as microRNAs) are more important than the stem cells in tissue-protective effects. Therefore, it's possible to consider SC-EVs as an alternative to stem cells in the treatment of PBI (Keshtkar et al., 2018; Sisa et al., 2019). Compared to stem cells, SC-EVs are postulated to be potentially being safer as they have lower amounts of membrane-bound proteins (such as major histocompatibility complex molecules) and lack the inability to directly form tumors (Riazifar et al., 2019). In addition, SC-EVs can carry nucleic acids (DNA, RNA, microRNA and non-coding RNA), proteins and lipids, and has the ability to cross biological barriers (e.g., blood-brain barrier), as well as high physicochemical stability and biocompatibility, making them an ideal delivery vehicle (Gamage and Fraser, 2021; Zhang et al., 2019; Zhang et al., 2021). SC-EVs could be genetically engineered to transport therapeutic protein or nucleic acid cargoes to target cells to ameliorate damage (Rädler et al., 2023).

Recently, the rapid advancement of SC-EVs has prompted multiple meta-analyses systematically evaluating preclinical studies on mature brain injury models (e.g., adult stroke, traumatic brain injury, and Parkinson's disease) (Shekari et al., 2021; Xylaki et al., 2023; Yang et al., 2023; Zhao et al., 2023). However, the field of PBI research currently lacks comprehensive systematic reviews and quality assessments of SC-EVs-based treatments. Although research on SC-EVs is burgeoning, its clinical translation remains hindered by insufficient evidence (Malhotra et al., 2020; Sisa et al., 2019; Thomi et al., 2019a). Therefore, we conducted this systematic review and meta-analysis to evaluate the efficacy and possible mechanisms of SC-EVs-based therapy in improving behavioral and pathological outcomes in animals with PBI. This study could clarify the limitations of current preclinical study designs and clinical translation, provide updated evidence for relevant trials, and lay the foundation for the potential clinical application of SC-EVs in children with PBI.

2. Methods

The review protocol was registered on Prospero, number CRD42024565466 (https://www.crd.york.ac.uk/prospero/). The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) was used to perform this meta-analysis (Moher et al., 2009).

2.1. Search strategy

We included only published animal studies reported in the English language from their inception until May 25th, 2025. The following electronic databases were searched: MEDLINE, Embase, Scopus, PubMed, ProQuest, and Web of Science Databases. The entire search strategy for all databases was presented in the Supplementary Table A. 1.

2.2. Eligibility criteria

Studies were included for analysis if they met the following criteria: (i) included animal models of PBI induced during prenatal, perinatal, and postnatal stages (e.g., hypoxia-ischemia models, inflammatory models, hypoxia models, middle cerebral artery occlusion models, etc.); (ii) used native or engineered extracellular vesicles (EVs) derived from stem cells (e.g., mesenchymal stem cells, neural stem cells, etc.) as an intervention; (iii) included a comparison between an untreated injury group and a SC-EVs treatment group; (iv) included measures of infarct size and brain water content as primary outcome measures and at least one of the following as secondary measures: neurobehavioral outcomes, apoptosis, neuronal numbers, remyelination, angiogenesis, or inflammatory response.

We excluded (i) studies that used animal models of mature brain injury and in vitro studies; (ii) studies that included non-stem cell derived EVs or combined with other interventions; (iii) reviews, study protocols, conference papers, and meta-analyses; (iv) studies not published in English; (v) studies without full text and or those lacking original data.

2.3. Study selection

The records were managed by Endnote 20. Duplicates were screened out by Endnote and unrecognized duplicates were manually removed by the evaluators. Two independent reviewers screened all articles according to the inclusion and exclusion criteria. Disagreements between reviewers were resolved through discussion with the third person in the team.

2.4. Data extraction

The following items were extracted from each included study: reference details (name of the paper and authors, published year, country), type of EVs (native or engineered), animal model (species, type and time of perinatal brain injury), functional outcomes, histopathological outcomes, and commonly measured brain injury markers. The mean and standard deviation (SD) of outcome indicators in the injured group and SC-EVs treatment group were extracted independently by two investigators. For articles with missing data, we contacted

the authors to provide additional data. Data for graphs were extracted using WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/) if only graphs were available. If the article did not provide SD, we calculated it by multiplying the standard error (SE) by the square root of the sample size or by the median and quartiles. When multiple assessment time-points of a single endpoint were reported, only the last time-point outcome was included. In addition, natural EVs intervention and engineered EVs intervention were treated as separate datasets in a study.

2.5. Quality assessment

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)'s risk of bias tool was used to assess the potential for bias in each study included in the review. Selection bias, performance bias, detection bias, attribution bias and reporting bias would be assessed by two independent evaluators. Disagreements were resolved by a third evaluators.

2.6. Subgroup analysis

To investigate whether the therapeutic efficacy of SC-EVs varies across different patient populations and intervention characteristics, we selected infarct size as the primary endpoint for subgroup analysis due to its objective and quantifiable nature. Our predefined subgroup analyses included the following variables: (1) Model type: preterm models (defined as injury induction before postnatal day 7 in rats and before postnatal day 9 in mice) and term models (Purcell et al., 2023); (2) EVs administration time: $<$ 24 h post-injury and $\geq$ 24 h post-injury; (3) Source of EVs: xenogeneic and allogeneic; (4) Type of EVs: bone marrow MSC-derived, placental MSC-derived and other cell type-derived; (5) Route of administration: intranasal, intracardiac, intraperitoneal and intracerebroventricular; (6) Total dosage: 10^7~10^8 particles, 10^8~10^9 particles and $> 10^9$ particles. For standardized dose comparison, we adopted the "therapeutic unit" concept, where one unit represents the EVs yield from $4*10^7$ human bone marrow-derived MSCs over 48 h (approximately $1.3–3.5*10^{10}$ particles/unit, containing 0.5–1.6 mg protein) (Kordelas et al., 2014). (7) EVs modification: natural EVs and engineered EVs. We assessed both between-subgroup statistical differences and within-subgroup pooled effect estimates.

2.7. Statistical analysis

Quantitative data were analyzed using Review Manager version 5.3 (RevMan, Cochrane Collaboration, North Europe) and Stata 15.1 (StataCorp, College Station, USA). We used random-effects inverse variance model to calculate the standardized mean difference (SMD) and 95 % confidence interval (CI). Heterogeneity was assessed using the I^2 statistic, with values exceeding 50 % indicating substantial heterogeneity. $P < 0.05$ was considered statistically significant. In addition, subgroup analyses were conducted to identify potential sources of heterogeneity, and sensitivity analysis was used to examine overall stability. Funnel plots, Egger's test and the trim-and-fill method were employed to check for any potential publication bias in the data.

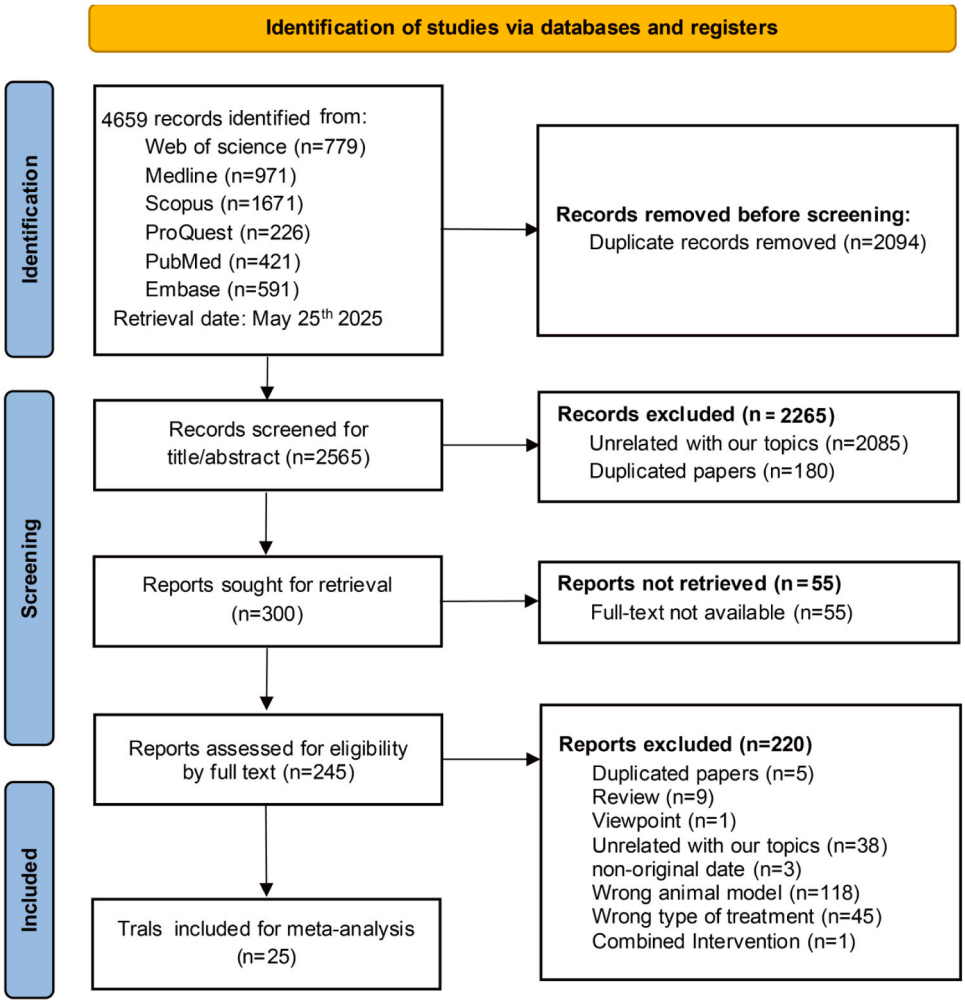

Fig. 1. PRISMA flow diagram for review and selection process of studies included in meta-analysis of stem cell derived extracellular vesicles in perinatal brain injury.

3. Result

3.1. Search results

We searched 4659 articles from 6 databases, and after excluding duplicates, 2565 were screened by title and abstract. Subsequently, 300 articles were screened by full text. The full text was not available for 55 articles. Two hundred and twenty of these articles were excluded due to animal model (n = 118) and intervention mismatch (n = 46), topic irrelevance (n = 38), no original data (n = 3), literature type mismatch (n = 10), and duplicate literature (n = 5). The final 25 studies were included in this systematic review. PRISMA flow diagram is shown in Fig. 1.

3.2. Study characteristics

Most studies used rodents (mice, n = 13; rats, n = 11), with only one employed fetal sheep. Brain injury models for both preterm (n = 14) and term (n = 11) births encompassed various types, such as hypoxic-ischemic model (n = 15), hypoxic-ischemic combined inflammatory model (n = 3), inflammatory model (n = 2), hypoxic model (n = 1), propofol-induced model (n = 1), middle cerebral artery occlusion model (MCAO) (n = 2), and preeclampsia (PE)-like animal model (n = 1). Most studies used ultracentrifugation to extract SC-EVs (n = 23), and SC-EVs' morphology and surface markers were identified by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and western blot (WB) (n = 16). The mean diameter range of the isolated SC-EVs was less than 200 nm. Fourteen studies used natural SC-EVs, and 11 used modified engineered SC-EVs. Bone marrow mesenchymal stem cells (MSCs) were the main source of SC-EVs (n = 14), and other sources included umbilical cord tissue MSCs (n = 8), placental MSCs (n = 1), neural stem cells (n = 1), and amniotic fluid stem cells (n = 1). Administration routes for SC-EVs included intranasal (n = 9), intracerebroventricular (n = 5), intraperitoneal (n = 6), intracardial (n = 4), and intravenous (n = 2). The dosage units varied across studies, with total protein amount (n = 10), cellular equivalent (n = 7), and particle number (n = 8) were used to quantify SC-EVs. Fifteen of the 25 studies performed a single injection of SC-EVs, and 10 studies administered multiple injections. In addition, SC-EVs were injected between 14 h before injury to 9 days post-injury, and the follow-up period ranged from 6 h to 125 days after injection in all studies. Only 7 of all studies reported the occurrence of adverse events, but none were related to SC-EVs treatment. Table 1 summarizes the characteristics of all studies (Chu et al., 2020; Drommelschmidt et al., 2017; Han et al., 2020; Kaminski et al., 2020; Kim et al., 2022; Labusek et al., 2023; Lawson et al., 2022; Li et al., 2022; Luo et al., 2022; Min et al., 2022; Ophelders et al., 2016; Pathipati et al., 2021; Shen et al., 2022; Shu et al., 2025; Sisa et al., 2019; Sun and Zhang, 2024; Sun et al., 2024; Thomi et al., 2019a; Thomi et al., 2019b; Tscherrig et al., 2024; Turovsky et al., 2022; Xiao et al., 2025; Xin et al., 2021; Xin et al., 2020; Xin et al., 2022).

3.3. Study quality

To assess the methodological quality of the study, we used SYRCLE's risk of bias tool for animal studies (Hooijmans et al., 2014). The details of the study quality are shown in Supplementary Fig.A.1. For most studies, sequence generation, baseline characteristics, and allocation concealment were not described in detail, with only two articles reported specific methods of sequence generation, and two reported allocation concealment. We judged 20 studies with random housing to be at low risk because of their identical husbandry conditions. Blinding of performance bias, detection bias, and attrition bias were reported in only a few of the studies described. In addition, although none studies provided protocol, they all reported all outcomes, resulting in a reporting bias of low risk. Other bias was considered to be unclear for all studies. Overall, no studies were judged to have a high risk of bias.

3.4. Primary outcomes

3.4.1. SC-EVs reduced infarct size and edema after PBI

The effect of SC-EVs on severity of neurological impairment is shown in Fig. 2. To comprehensively assess the severity of neurological impairment, we employed both infarct size measurements to evaluate cerebral ischemia extent and brain water content measurements to assess tissue edema (Gerriets et al., 2004). Across 14 studies involving 199 animals, changes in infarct size after SC-EVs administration were reported (Fig. 2A), with 9 studies reporting infarct volume and 5 studies reporting tissue loss. The results indicated that SC-EVs significantly reduced infarct size compared to the injured group (SMD=2.04; 95 % CI 1.30, 2.78; $P < 0.001$; I^2=68 %). In addition, we evaluated the effect of SC-EVs on brain edema after PBI (Fig. 2B). Among 6 studies comprising 88 animals that were evaluated using brain water content, the results showed that SC-EVs significantly reduced brain tissue edema after PBI (SMD=2.22; 95 % CI 1.09, 3.35; $P < 0.001$; I^2=67 %).

3.5. Secondary outcomes

3.5.1. SC-EVs promoted neurobehavioral recovery after PBI

A total of 177 animals were subjected to behavioral tests in 8 studies, as shown in Fig. 3. For learning and memory function, 6 studies conducted the Morris water maze test, measuring escape times (Fig. 3A). For motor and sensory function, 3 studies performed Negative geotaxis test (Fig. 3B). The results showed that SC-EVs significantly improved cognitive function (SMD=1.45; 95 % CI 0.70, 2.20; $P = 0.0002$; I^2=63 %) and motor function (SMD=5.60; 95 % CI 3.91, 7.30; $P < 0.001$; I^2=46 %) after PBI.

3.5.2. SC-EVs improved apoptosis, neuroprotection, remyelination, and angiogenesis after PBI

Apoptosis was assessed in 14 studies using terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) as markers (Fig. 4A). Meta-analysis showed that SC-EVs significantly decreased cell apoptosis in whole brain tissue (SMD=2.76; 95 % CI 1.91, 3.61; $P < 0.001$; I^2=73 %). Five studies counted the number of neurons using immunostaining of neuronal nuclei antigen (NeuN) as a neuronal marker and Nissl staining (Fig. 4B). Meta-analysis showed that SC-EVs increased neuronal number (SMD=-1.75; 95 % CI −2.86, −0.64; $P = 0.002$; I^2=57 %). Six studies detected myelin regeneration using the oligodendrocyte marker myelin basic protein (MBP) (Fig. 4C). SC-EVs showed significant increases in the remyelination after PBI (SMD=-1.21; 95 % CI −1.67, −0.75; $P < 0.001$; I^2=0 %). Three studies assessed angiogenesis using the vascular endothelial cell marker platelet endothelial cell adhesion molecule 1(CD31) (Fig. 4D). The results indicated that SC-EVs could promote angiogenesis after PBI (SMD=-0.77; 95 % CI −1.38, −0.16; $P = 0.01$; I^2=0 %).

3.5.3. SC-EVs suppressed microglia activation, astrogliosis and the release of pro-inflammatory factors after PBI

The mechanisms of SC-EVs immunomodulation are demonstrated in Fig. 5 and Fig. 6. Eleven studies evaluated ionized calcium binding adaptor molecule 1 (Iba-1) as a marker of microglia activation (Fig. 5A), and the results showed that SC-EVs significantly reduced microglia activation after PBI (SMD=1.74; 95 % CI 1.11, 2.37; $P < 0.001$; I^2=61 %). Similarly, four studies assessed glial fibrillary acidic protein (GFAP) as a marker of astrogliosis (Fig. 5B), and meta-analysis indicated that SC-EVs significantly inhibited astrogliosis (SMD=0.91; 95 % CI 0.43, 1.38; $P = 0.0002$; I^2=0 %). Eleven studies involving 194 animals reported the release of pro-inflammatory factors after PBI (Fig. 6A-C), with two studies divided into two groups based on SC-EVs modification. Ten studies entries reported that SC-EVs significantly reduced tumor necrosis factor alpha (TNF-α) release (SMD=2.62; 95 % CI 1.49, 3.76; $P < 0.001$; I^2=85 %). Nine studies entries showed that SC-EVs reduced interleukin-1β (IL-1β) release (SMD=2.14; 95 % CI 1.04, 3.23;

Table 1
Characteristics of the included studies.

Study	Strain and Species	Brain injury model and time	Isolation methods	Characterization methods	Diameter range	EVs modification	Source and Type	EVs administration time	Route and Total doses	End time points	Reported Complications and Side-Effects
Chu X (2020)	C57BL/6 J mice	HI, preterm, P7	UC and SEC	TEM;WB;NTA	NA	miR−7b−5p	mice BM-MSCs	24 h after HI	intracardial, 1.5×10^8 particles, 1 doses	P10, P42	NA
Drommelschmidt K (2017)	Wistar rat	LPS-induced model, preterm, P3	UC and PEG	NTA;WB	peak 110 nm	Native	hBM-MSCs	3 h before and 24 h after LPS injection	intraperitoneal, 1×10^8 cell equivalents/kg, 2 doses	P5, P11, P125	one died (LPS vehicle)
Han J (2021)	C57BL/6 J mice	HI, term, P9	UC	TEM;WB;NTA	30 ~100 nm	miR−410	hUC-MSCs	14 h before HI, before exposure to hypoxia, removed from the hypoxic chamber, and 3 h after hypoxia	intraperitoneal, 2×10^5 cell equivalents, 4 doses	6 h after HI	NA
Kaminski N (2020)	C57BL/6 J mice	HI, term, P9	UC and PEG	TEM;WB;NTA	NA	Native	hBM-MSCs	24, 72, and 120 h after HI	intraperitoneal, 1×10^5 cell equivalents/g, 3 doses	P16	five animals died (2 vehicle, 1 PL-EV, 2 MSC-EV)
Kim YE (2022)	Sprague-Dawley rat	E. coli Meningitis insulted model, term, P11	UC	NTA; SEM; TEM; WB	peak 100 nm	Native	hWJ-MSCs	6 h after induction of meningitis	intracerebroventricular, 1×10^5 cell equivalents, 1 dose	P17	No mortality
Labusek N (2023)	C57BL/6 J mice	HI, term, P9	UC and PEG	NTA; ImFC	peak 109.6, 116.3 nm	Native	hBM-MSCs	1,3,and 5d after HI	intranasal, 1×10^5 cell equivalents/g, 3 doses	P16	five animals died
Lawson A (2022)	CD1 mice	HI, term, P9	UC	TEM;WB;NTA	peak 152, 208 nm	Native	mice Neural stem cell	30 min and 24 h after HI	intranasal, 8×10^9 particles, 2 doses	P10	NA
Li P (2022)	CD1 mice	hypoxia model, preterm, P3	UC	TEM;WB;NTA	60 ~ 150 nm	Native	hAFS	2 h after the last hypoxia	intravenous, 50 μg, 1 dose	P34	NA
Luo H (2022)	C57BL/6 J mice	HI, preterm, P7	UC	TEM;WB;NTA	100 ~ 200 nm	miR−93	mice BM-MSCs	after hypoxia	intranasal, 2×10^9 particles, 1 dose	P14	survival rate
Min W (2022)	Sprague-Dawley rat	HI, term, P7	UC	TEM;WB;NTA	40 ~ 100 nm	miR−124–3p	mice BM-MSCs	2d after HI	intraventricular	P14	NA
Ophelders DR (2016)	Sheep	HI, preterm, GA106	PEG	NTA;WB	peak 110 nm	Native	hBM-MSCs	1 h and 4d after UCO	intravenous, 2×10^7 cell equivalents, 2 doses	7d after UCO	four animals died
Pathipati P (2021)	C57BL/6 J mice	tMCAO, term, P9	UC and EQ	NTA;WB	30–200 nm	Native	mice BM-MSCs	after tMCAO	intracerebroventricular (1 μg) or intranasally(5 μg), 1 dose	2 h, 18 h, 72 h after tMCAO	NA
Shen M (2022)	BALB/c mice	HI, preterm, P7	UC	TEM;WB;NTA	peak 155 ± 2.8 nm	miR−410	mice BM-MSCs	after HI	intracerebroventricular, 5×10^4 cell equivalents, 1 dose	P10, P49	10 % mortality rate of HIBD model
Sisa C (2019)	C57BL/6 J mice	HI, term, P9	UC	NTA; EM; FACS; WB	30 ~ 1000 nm	Native	hBM-MSCs	after hypoxia	intranasal, 1.25×10^9 particles, 1 dose	P11	NA
Sun J (2024)	Sprague-Dawley rat	PE-like animal model, preterm, GD14	UC	TEM;WB;NTA	peak 97 nm	miR−144	hUC-MSCs	GD14-GD19	intraperitoneal, 1.55×10^{10} particles, 6 doses	GD18, GD20	NA
Shu J (2025)	Sprague-Dawley rat	MCAO, preterm,P3	UC	NA	NA	miR−653–3p	hBM-MSCs	2 h after MCAO	intracerebroventricular, 10 μl, 1 dose	P24	the mortality rate 16.6 %
Sun W (2024)	Sprague-Dawley rat	Propofol-induced	NA	TEM; qRT-PCR; WB	82 ± 20 nm	NUFIP1	hUC-MSCs	P7-P13	intraperitoneal, 7×10^8 particles, 7 doses	P60; P65; P66	NA

(*continued on next page*)

Table 1 (*continued*)

Study	Strain and Species	Brain injury model and time	Isolation methods	Characterization methods	Diameter range	EVs modification	Source and Type	EVs administration time	Route and Total doses	End time points	Reported Complications and Side-Effects
		model, term, P7									
Thomi G_1 (2019)	Wistar rat	HI, preterm, P2	UC	EM; Exo-Check Antibody Array	16.34 ~ 87.18 nm	Native	hWJ-MSCs	before the cauterization of the left common carotid	intranasal, 50 mg/kg, 1 dose	P3	NA
Thomi G_2 (2019)	Wistar rat	HI, preterm, P2	UC	Exo-Check Antibody Array; EM;WB	peak 34.34 nm	Native	hWJ-MSCs	before the cauterization of the left common carotid	intranasal, 50 mg/kg, 1 dose	P3, P11, P30-P34	survival rate
Tscherrig V (2024)	Wistar rat	WMI, preterm, P2	UC and SEC	NA	NA	miRNAs	hWJ-MSCs	24 h after HI	intranasal, 8×10^8 particles/10 g, 1 dose	P4, P11	NA
Turovsky EA (2022)	Wistar rat	HI, term, P7	UC	TEM;WB;NTA	20 ~ 360 nm	Native	human postpartum placenta MSCs	1 h after HI and P8-P16	intranasal, 1.6 $\pm$ 0.2 $\times 10^{11}$ particles/mL, 20 μL, 10 doses	P47, P67	NA
Xiao S (2025)	Sprague-Dawley rat	HI, term, P7	UC	TEM;WB;NTA	40–150 nm	Native	hUC-MSCs	14 h before HI, before exposure to hypoxia, removed from the hypoxic chamber, and 3 h after hypoxia	intraperitoneal, 100 μg, 4 doses	P8, P77	NA
Xin D_1 (2020)	C57BL/6 J mice	HI, preterm, P7	UC and SEC	TEM;WB;NTA	60 ~ 160 nm	miR−21a−5p	BM-MSCs	24 h after HI	intracardial, 100 μg, 1 dose	P10, P12, P21, P42-P47	NA
Xin D_2 (2021)	C57BL/6 J male mice	HI, preterm, P7	UC	TEM;WB;NTA	60 ~ 120 nm	Native	mice BM-MSCs	24 h after HI	intracardial, 100 μg, 1 dose	P10, P21, P35	NA
Xin D_3 (2022)	C57BL/6 J mice	HI, preterm, P7	UC and SEC	TEM;WB;NTA	60 ~ 120 nm	miR−21a−5p	mice BM-MSCs	24 h after HI	intracardial, 100 μg, 1 dose	P10	NA

Abbreviations: *HI* hypoxic-ischemic brain injury, *tMCAO* transient middle cerebral artery occlusion, *PE* preeclampsia, *WMI* white matter injury, *LPS* lipopolysaccharide, *UC* ultracentrifuging, *PEG* polyethylene glycol, *SEC* size exclusion chromatography, *EQ* ExoQuick-TC, *TEM* transmission electron microscopy, *SEM* scanning electron microscopy, *EM* electron microscopy, *WB* western blot, *NTA* nanoparticle tracking analysis, *ImFC* imaging flow cytometry-based analyses, *FACS* fluorescence activating cell sorter, *qRT-PCR* quantitative real time polymerase chain reaction, *miR* microRNA, *BM-MSCs* bone marrow mesenchymal stem cells, *hWJ-MSCs* human wharton's jelly mesenchymal stromal cells, *hUC-MSCs* human umbilical cord mesenchymal stem cells, *hAFS* human amniotic fluid derived stem cells, *UCO* umbilical cord occlusion, *P* postnatal day, *h* hour, *d* day, *GD* gestation day, *GA* gestational age, *EVs* extracellular vesicles, *NA* not available.

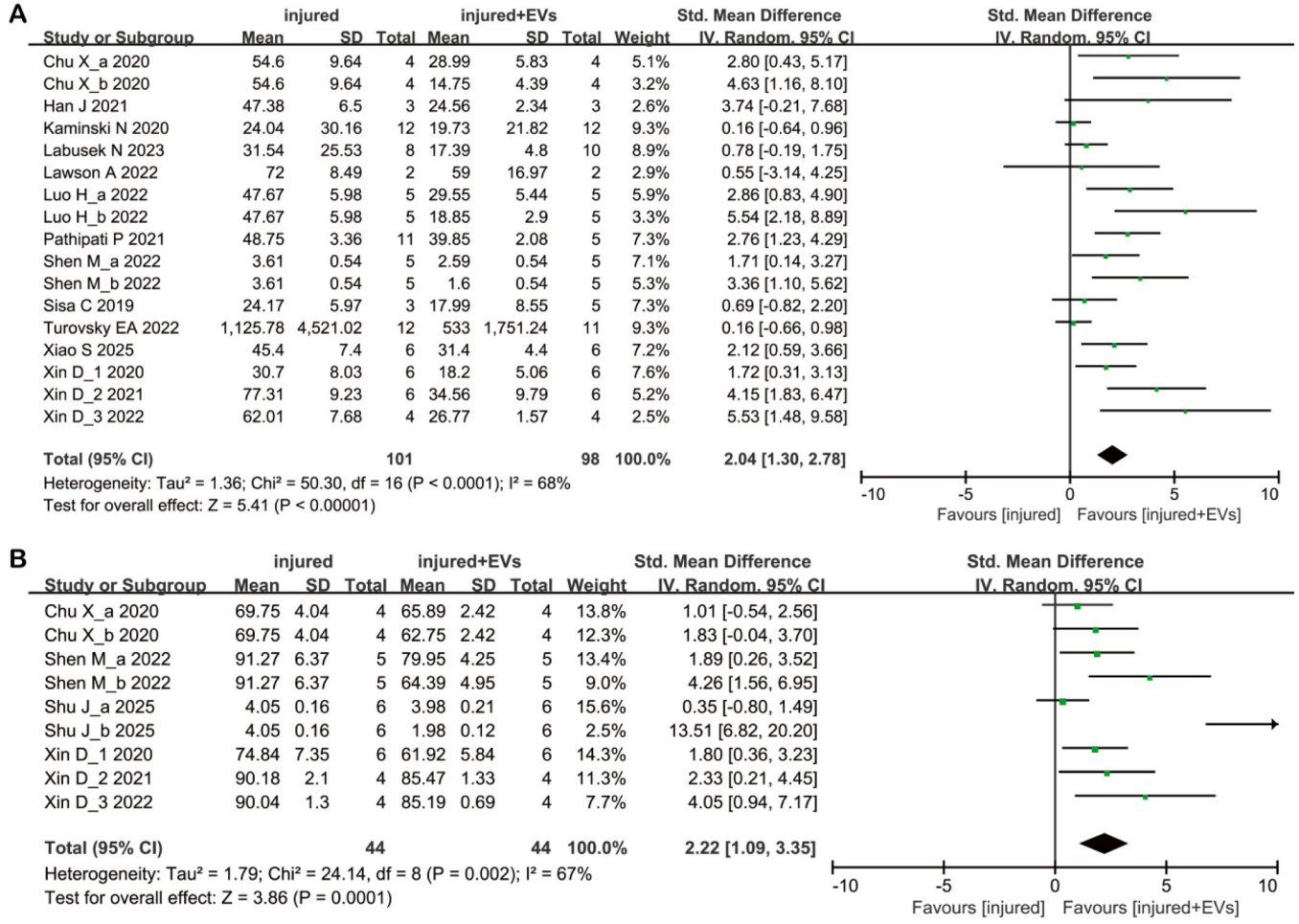

Fig. 2. Forest plot of the effect of stem cell derived extracellular vesicles on neurological severity after perinatal brain injury. (A) Infarct size (B) Brain water content. *EVs* extracellular vesicles, *CI* confidence interval.

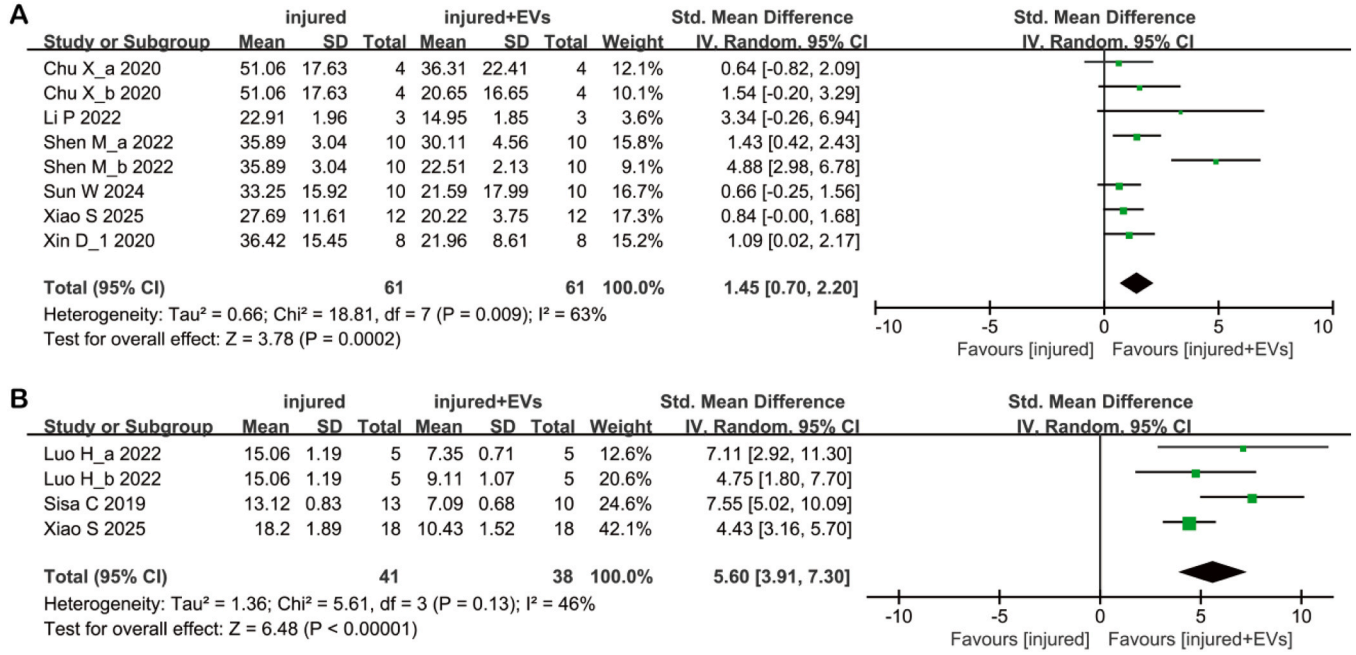

Fig. 3. The forest plot of the effect of stem cell derived extracellular vesicles on neurobehavioral recovery after perinatal brain injury. (A) Morris water maze test-escape time (B) Negative geotaxis test. *EVs* extracellular vesicles, *CI* confidence interval.

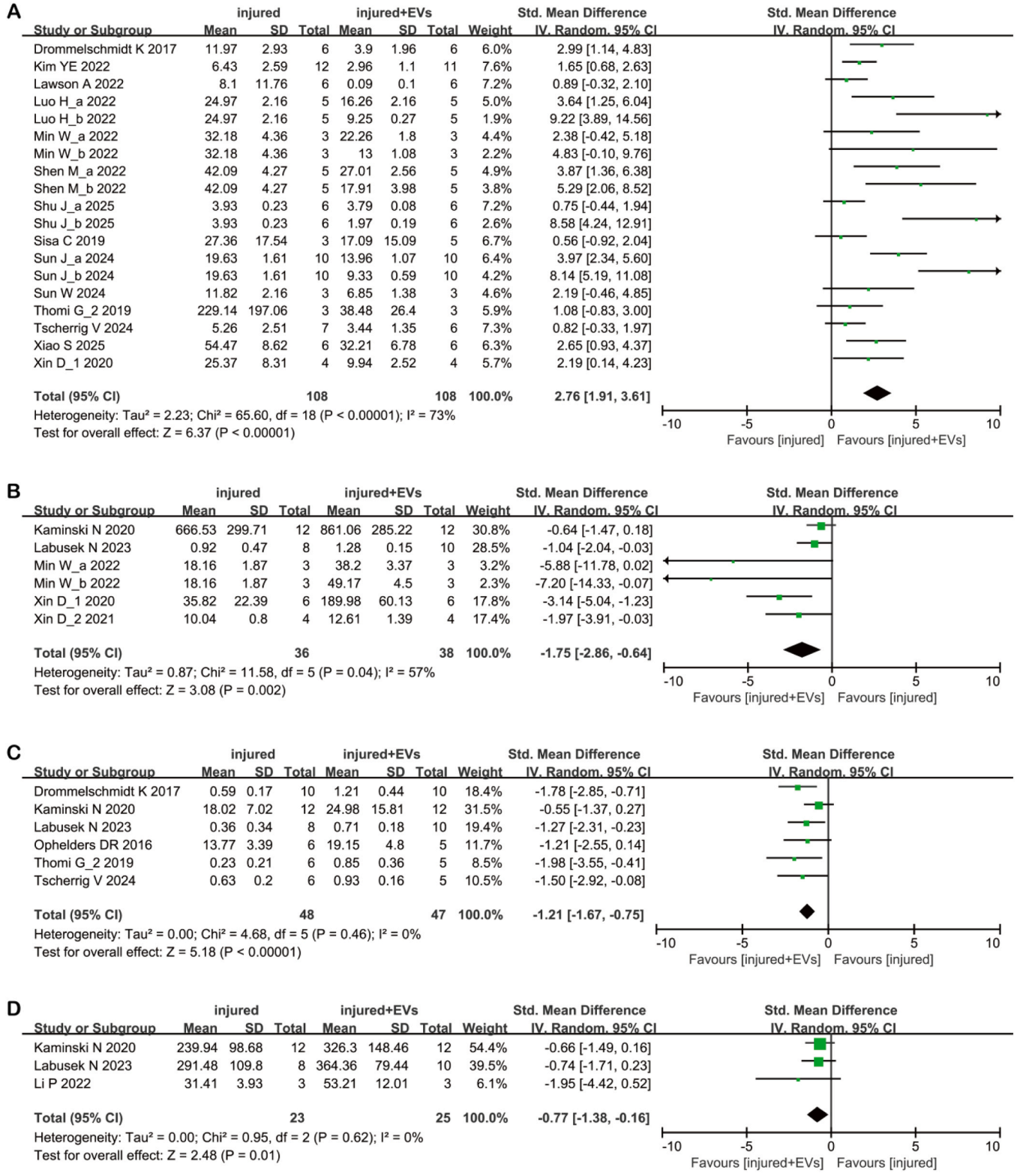

Fig. 4. The forest plot of the effect of extracellular vesicles on apoptosis, neuronal protection, remyelination, angiogenesis after perinatal brain injury. (A) Apoptotic rate. (B) Number of neurons (C) Oligodendrocyte number (D) Vessel densities. *EVs* extracellular vesicles, *CI* confidence interval.

$P = 0.0001$; I^2=81 %). Six studies entries reported that SC-EVs reduced interleukin-6 (IL-6) release (SMD=1.40; 95 % CI 0.19, 2.61; $P = 0.02$; I^2=82 %). In brief, SC-EVs could suppress neuroinflammation and reduce the release of pro-inflammatory factors.

3.6. Subgroup analysis

We performed subgroup analysis of infarct size shown in Table 2. We stratified by modeling time, which showed that SC-EVs had a significantly better effect size for preterm models compared to term models ($P = 0.0008$, Supplementary Fig.A.2). The effect size of engineered SC-

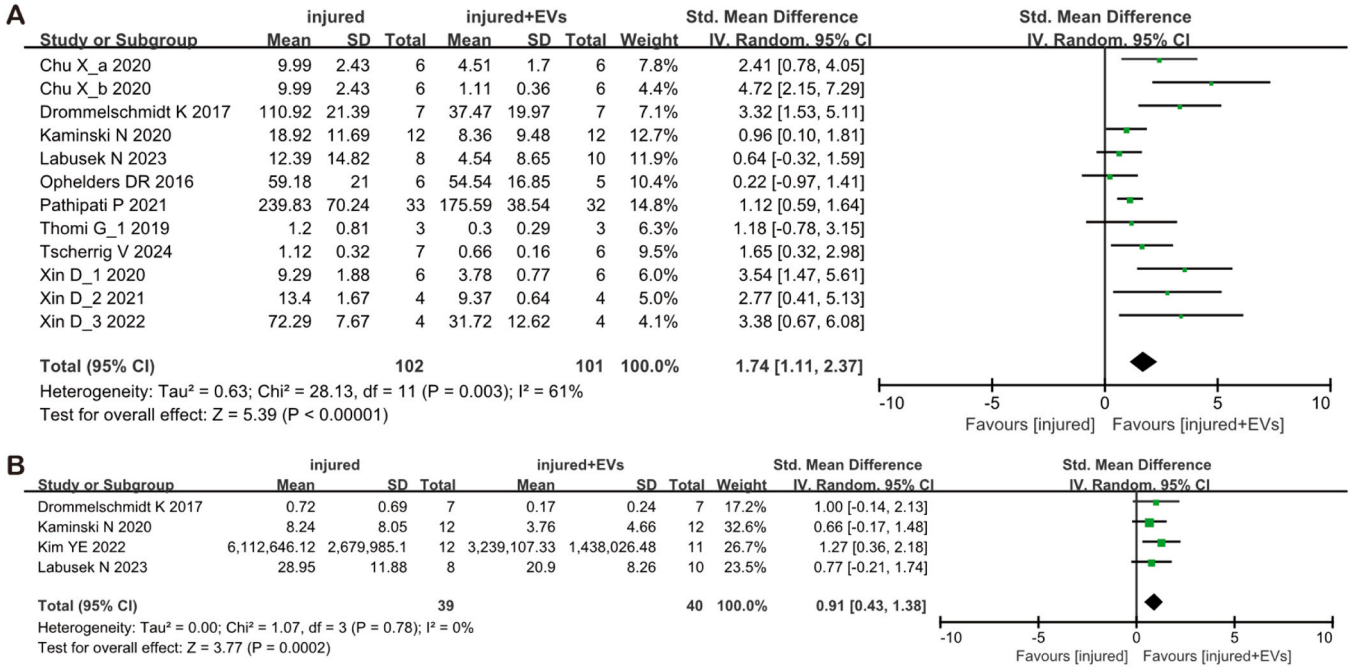

Fig. 5. Forest plot of the effect of stem cell derived extracellular vesicles on immunoregulation after perinatal brain injury. (A) Microglia activation (B) Astrogliosis. *EVs* extracellular vesicles, *CI* confidence interval.

EVs was significantly higher than that of natural SC-EVs in terms of whether the SC-EVs were modified ($P = 0.006$, Supplementary Fig.A.3). Among the sources of stem cells used for SC-EVs, the effect size of allogeneic was statistically different and superior to xenogeneic ($P < 0.001$, Supplementary Fig.A.4). In addition, there were no significant differences in the estimation of effect size by stem cell type, route, total dose and time of administration ($P = 0.52$, Supplementary Fig.A.4, $P = 0.27$, Supplementary Fig.A.5; $P = 0.89$, Supplementary Fig.A.6; $P = 0.38$, Supplementary Fig.A.7).

3.7. Sensitivity analysis

Given the significant heterogeneity of the included studies (I^2 >50 %), we conducted sensitivity analyses to ensure stability of the overall effect size. We performed sensitivity analyses by omitting each study in turn and recalculating the combined effect size for the remaining studies. The recalculated pooled results did not significantly change, indicating that there was no outlying study that significantly influenced the overall results (Fig. 7A and Supplementary Fig.A.8–9).

3.8. Publication bias

We also tested publication bias and generated funnel plots for infarct size. The results showed asymmetry in the funnel plots for infarct size (Fig. 7B) and the Egger's test suggested the same comments ($P < 0.05$). Subsequently, we added missing studies and recalculated the pooled effect values using the trim-and-fill strategy (Fig. 7C). The overall results did not change significantly, indicating no "missing" studies.

4. Discussion

4.1. Summary of findings

In this systematic review, we analyzed 25 preclinical studies to summarize the therapeutic effects of SC-EVs in animal models of PBI. We focused on the effects of SC-EVs on the immature brain injury, exploring both brain microstructural changes and underlying mechanisms. The findings showed that SC-EVs significantly reduced infarct size and tissue edema, and promoted the recovery of cognitive and motor functions after PBI. The mechanism of SC-EVs may involve the inhibition of apoptosis, an increase in neuronal number, the promotion of remyelination and angiogenesis, and the reduction of glial cell activation and release of pro-inflammatory factors. However, given the limited number of included studies included, further research is needed to fully elucidate the effects and mechanisms of SC-EVs in PBI.

4.2. Possible mechanisms of SC-EVs in models

PBI is mainly caused by triggering factors such as hypoxia-ischemia, inflammation and preterm delivery marked by neuronal excitotoxicity, cellular apoptosis and inflammation induced by microglial activation (Novak et al., 2018). The response to brain injury in immature versus adult brains could differ significantly, with the maturation state of the brain may play a key role in determining the post-injury outcomes (Semple et al., 2013). Compared to the adult brain injury, immature brain injury results in significant reductions in cortical and hippocampal volumes, attributed to both the loss of infarcted tissue and impaired development of the surrounding tissue (Li et al., 2011). Moreover, apoptosis, immunoinflammation, and oxidative stress are much more activated after immature brain injury than in the adult brain (Campbell et al., 2007; Zhu et al., 2005). Therefore, targeting the specific pathological processes of immature brain injury is important for exploring its potential therapies. With the potential of SC-EVs in animal models of immature brain injury, there has been a progressive shift towards exploring the mechanisms underlying its efficacy. Studies have shown that SC-EVs could reduce early microglia activation and astrocyte reactive proliferation after PBI (Kaminski et al., 2020; Kim et al., 2022; Labusek et al., 2023; Pathipati et al., 2021), the release of pro-inflammatory factors TNFα and IL-1β (Drommelschmidt et al., 2017; Luo et al., 2022), and neuronal apoptosis (Chu et al., 2020; Sisa et al., 2019), exerting immunomodulatory and anti-apoptotic effects. Over time, SC-EVs promoted neuron generation (Kaminski et al., 2020; Li et al., 2022; Xin et al., 2020) and remyelination (Drommelschmidt et al., 2017; Kaminski et al., 2020; Ophelders et al., 2016) in various brain regions, improved brain microstructure, and ultimately enhanced cognitive (Li et al., 2022; Shen et al., 2022; Sun et al., 2024) and motor

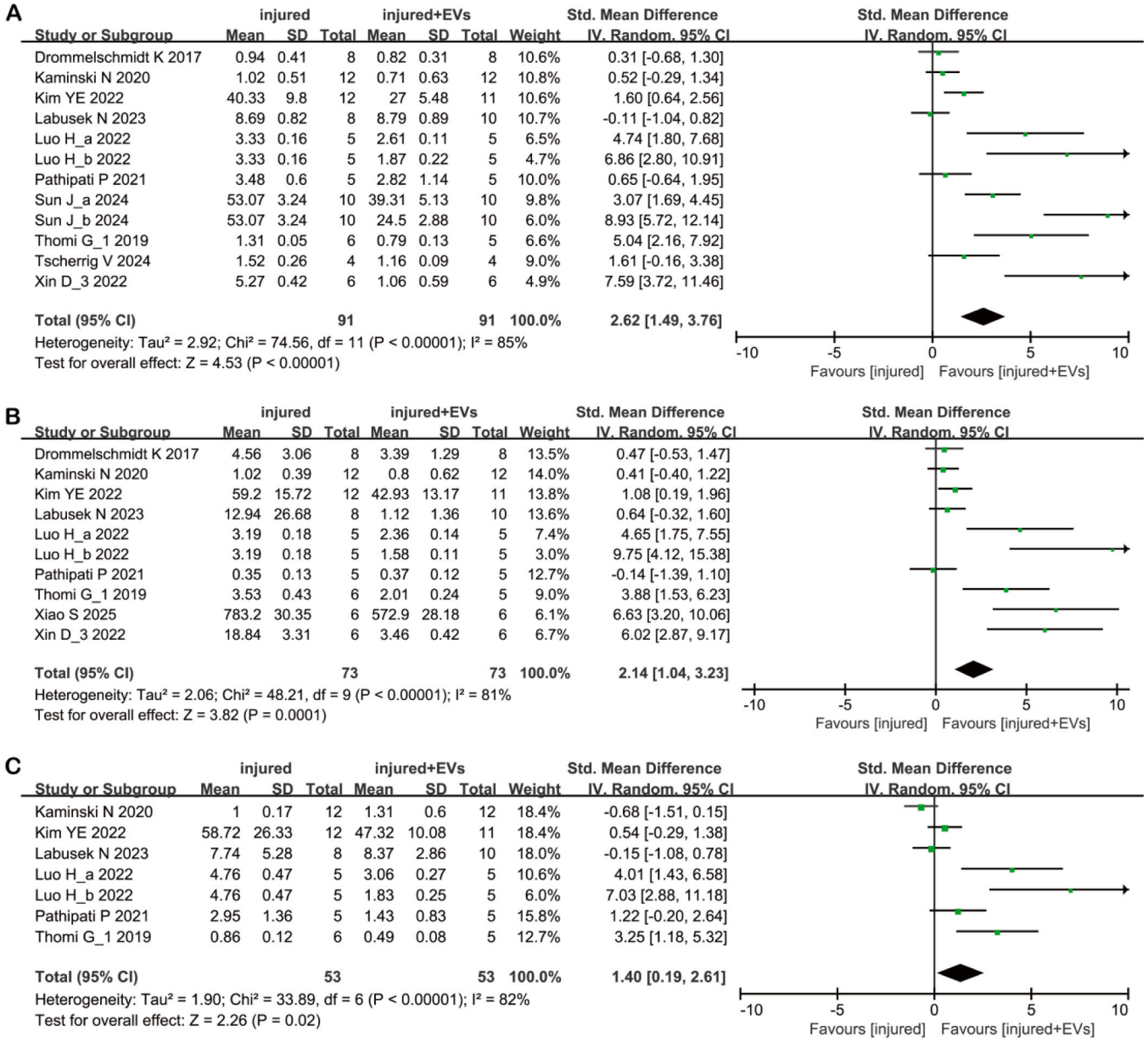

Fig. 6. Forest plot of the effect of stem cell derived extracellular vesicles on pro-inflammatory factor release after perinatal brain injury. (A) TNF-α (B) IL-1β (C) IL-6. *EVs* extracellular vesicles, *CI* confidence interval, *TNF-α* tumor necrosis factor alpha, *IL-1β* interleukin-1β, *IL-6* interleukin-6.

functions (Luo et al., 2022; Sisa et al., 2019) after PBI. SC-EVs were also observed to promote the proliferation of endothelial cells, thereby enhancing blood vessel density (Kaminski et al., 2020; Labusek et al., 2023; Li et al., 2022) and blood-brain barrier function (Gussenhoven et al., 2019). Our meta-analysis suggests that SC-EVs could improve neurological function in animal models of PBI by inhibiting neuroinflammation and apoptosis, which in turn promotes neuron generation, remyelination and angiogenesis, and that these responses coincide with biochemical changes specific to immature brain injury.

4.3. Interpretation of the subgroup analysis

In order to investigate the factors affecting the effectiveness of SC-EVs, we conducted subgroup analyses, focusing on infarct size. We found that the preterm animal models with brain injury responded better to SC-EVs compared to the term models. This difference might be attributed to the varying injury patterns between preterm and term infants (van Tilborg et al., 2018). In preterm infants, perinatal hypoxia-ischemia mainly affects oligodendrocytes, resulting in significant white matter injury, while in term infants, hypoxia-ischemia mainly affects the basal ganglia and thalamus, leading to grey matter injury (Hamdy et al., 2020; Yıldız et al., 2017). The differential regions of injury could influence the efficacy of SC-EVs. Although detailed descriptions of infarct regions were lacking in most of the included studies, SC-EVs have potential as a therapy for white matter injury in preterm infants.

The source of the stem cells from which the SC-EVs were extracted significantly affected the therapeutic efficacy of SC-EVs. SC-EVs derived from rodents showed better performance compared to those derived from humans, possibly due to better immunocompatibility of allogeneic SC-EVs. This provides essential and necessary evidence-based data for the use of homologous SC-EVs in future clinical trials (Paton et al., 2022). Stem cell type did not significantly affect SC-EVs' efficacy in this study and most including studies have primarily utilized EVs derived from MSCs. These MSCs are predominantly sourced from two tissue types: BM-MSCs and placental MSCs. While BM-MSCs derived EVs

Table 2
Subgroup analysis of infarct size.

Categories	Number of study	Number of animal	SMD (95 % CI)	*P* value	Heterogeneity test			Subgroup analysis *P* value
					Q Statistic	I^2	*P* value	
Term vs. Preterm model								
Preterm	9	88	2.98 [2.10, 3.87]	< 0.00001	11.03	27 %	0.20	0.0008
Term	8	111	1.01 [0.28, 1.74]	0.03	15.99	56 %	0.03	
EVs administration time								
< 24 h after HI	9	86	2.29 [1.48, 3.11]	< 0.00001	11.33	29 %	0.18	0.38
≥ 24 h after HI	8	113	1.70 [0.67, 2.73]	0.001	27.05	74 %	0.0003	
Source of EVs								
Xenogenic	6	91	2.80 [2.06, 3.54]	0.09	8.56	42 %	0.13	< 0.0001
Allogeneic	11	108	0.69 [0.05, 1.33]	< 0.00001	12.37	19 %	0.26	
Type of EVs								
BM-MSCs	13	154	2.30 [1.42, 3.17]	< 0.00001	39.69	70 %	< 0.0001	0.52
Placenta Derived MSCs	3	41	1.46 [−0.40, 3.33]	0.12	7.22	72 %	0.03	
Neural stem cells	1	4	0.55 [−3.14, 4.25]	0.77	NA	NA	NA	
Route of administration								
Intranasal	7	89	1.56 [0.48, 2.63]	0.005	19.76	70 %	0.003	0.27
Intracerebroventricular	2	20	2.32 [0.75, 3.90]	0.004	1.39	28 %	0.24	
Intracardial	5	48	3.20 [1.82, 4.58]	< 0.00001	6.36	37 %	0.17	
Intraperitoneal	3	42	1.46 [−0.41, 3.34]	0.13	7.33	73 %	0.03	
Total doses								
10^7 ~10^8 particles	2	20	2.32 [0.75, 3.90]	0.004	1.39	28 %	0.24	0.89
10^8 ~10^9 particles	6	80	1.87 [0.61, 3.14]	0.004	17.78	72 %	0.003	
> 10^9 particles	9	99	2.18 [1.02, 3.34]	0.0002	27.98	71 %	0.0005	
EVs modification								
Natural EVs	14	171	1.68 [0.97, 2.39]	< 0.00001	16.46	64 %	0.0005	0.006
Engineered EVs	3	28	4.17 [2.52, 5.82]	< 0.00001	1.20	0 %	0.55	

Abbreviations: *HI* hypoxic-ischemic brain injury, *EVs* extracellular vesicles, *BM-MSCs* bone marrow mesenchymal stem cells, *SMD* standardized mean difference, *CI* confidence interval.

demonstrated a larger effect size in our analysis, this discrepancy likely reflects selection bias in research rather than a genuine biological advantage. The limited diversity in EVs sources may stem from the relative ease of isolating these cell types in laboratory settings, as well as researchers' preferential use of certain starting materials (Padinharayil et al., 2024). Therefore, future studies should prioritize standardized comparative research with balanced sample sizes to more objectively evaluate the therapeutic potential of SC-EVs from different cell types.

The dose, route and timing of administration are other factors that influence on the efficacy of SC-EVs in PBI. Our analysis, which standardized SC-EVs by particle count, revealed that the high dose injection group (>10^9 particles) showed slightly better effects compared to medium (10^8~10^9 particles). Sun J et al. explored the optimal concentration for SC-EVs and found that the high-dose group ($3.1*10^{10}$ particles/mL) had the best results in treating PE-like animal model (Sun and Zhang, 2024). However, possibly due to imprecise results after converting to particle counting using the concept of the "therapeutic unit" suggested by Kordelas et al. to standardize the doses of SC-EVs, future research may consider a total dose of at least 10^9 particles to further investigate SC-EVs' efficacy (Kordelas et al., 2014). Regarding the route of MSCs injection, Huang et al. found that intrathecal injection was most effective, but there was no experimental study compared the effects of SC-EVs in different routes (Huang et al., 2023). One included study compared intranasal and intracerebroventricular administration, demonstrating that the distribution patterns of SC-EVs in the brain were similar and recommended the intranasal route due to its non-invasive nature and physiological advantages (Pathipati et al., 2021). However, it did not further compare the therapeutic efficacy between these two routes. Additionally, current subgroup analyses remain insufficient to determine the optimal administration route, as the results may be confounded by variations in dosage and treatment frequency. Future studies should include direct comparisons of SC-EVs efficacy across different delivery routes to address this gap. Moreover, administering SC-EVs less than 24 h after injury or 24 h later did not result in a significant difference in cerebral infarct size. Therefore, further experimental studies are required to determine the optimal therapeutic time window for SC-EVs administration.

To improve the therapeutic potential of SC-EVs, some studies have focused on enhancing its efficacy and overcoming limitations such as low bioactivity, weak targeting, and rapid clearance in vivo by loading therapeutic cargoes (e.g., miR-21a-5p, miR-7b-5p, and miR-410) via artificial technique (de Abreu et al., 2020; Shen et al., 2022; Xin et al., 2022). Thus, we assessed the therapeutic potential of SC-EVs by categorizing them based on their modification. The results indicated that genetically engineered modified SC-EVs had significantly better effects on neurological function and brain microstructure after PBI compared to natural SC-EVs. Strong therapeutic potential and specific targeting of engineered EVs could offer substantial benefits, particularly in tailoring therapies to individual needs. which holds promise for enhancing neonatal clinical rehabilitation strategies in the future.

4.4. Challenges and potential strategies on the path to clinic

The potential of SC-EVs therapy is now evident with numerous EVs-based products undergoing clinical trials. However, transitioning SC-EVs therapy from preclinical studies to clinic encounters various challenges in PBI fields. The heterogeneity of SC-EVs underlies the significant challenges related to its exploitation. Current considerations include the following: (a) Standardized quality control of SC-EVs. There is a wide variation in SC-EVs' purification and storage methods, and a lack of consistent production standards, which could affect its size, structure and biofunction (Brennan et al., 2020). To mitigate this influence, the latest Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines and Good Manufacturing Practice (GMP) regulations should be followed wherever possible, and the development of a decision-making tool for quality control of SC-EVs, such as EV decision-making grid (EV-DMG) is recommended (Loria et al., 2024; Welsh et al., 2024). (b) Scale-up production. Large-scale production of SC-EVs is hampered by the limited proliferative capacity of donor cells and the isolation method with low recovery rate, poor purity, and time-consuming process (Richards et al., 2023). Potential solutions include using dynamic culture methods (e.g. bioreactors), selective stem cell sources (e.g. immortalized cells), and tangential flow filtration (Busatto et al., 2018; Kimiz-Gebologlu and Oncel, 2022). (c)

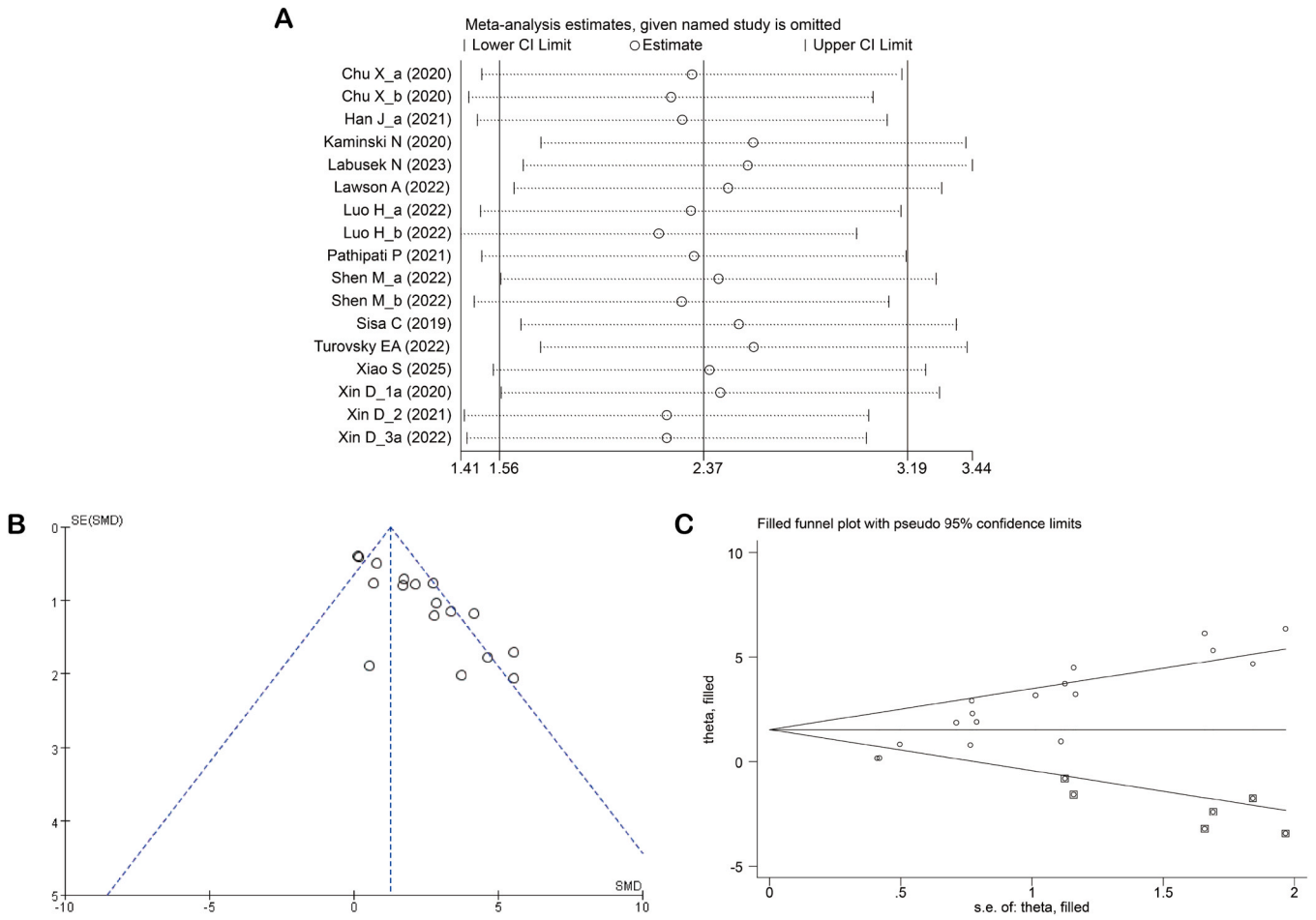

Fig. 7. Sensitivity analysis and evaluation of publication bias. (A) Sensitivity analysis of the studies included in infarct size. (B) Funnel plots for infarct size to evaluate publication bias. (C) Trim-and-fill method was used to evaluate the missing studies in infarct size. *SMD* standardized mean difference, *CI* confidence interval, *SE* standard error.

Batch-to-batch inconsistency. The lack of industry-standard quality specifications leads to non-reproducibility of SC-EVs products, and the use of cell culture and the isolation methods suitable for large-scale applications could solve this issue. Furthermore, uniformity and openness of procedures and reporting could be ensured by publishing sufficient information about the separation and characterization of SC-EVs in databases like EV-TRACK or ExoCarta. (d) Insufficient regulatory control. There is currently no standardized scheme or policy for the regulation of SC-EVs use in PBI fields. Efforts should be made to facilitate regulatory approval (e.g., the EMA Priority Medicines Scheme and FDA breakthrough therapy) for SC-EVs products (Paton et al., 2025).

Besides the difficulties of SC-EVs product development, the safety and specific efficacy of SC-EVs products are ongoing challenges for PBI field. It is important to report the adverse events and the potential toxicity in both preclinical and clinical studies, as this is the first step in the clinical translation of SC-EVs. There are no registered phases III and IV clinical trials of SC-EVs in CNS diseases, which requires more studies to specify mechanisms of action (MoA) and treatment regimens. Our work could provide valuable insights for future clinical studies in PBI fields to propel the transition from preclinic to clinic. Once the limitations in quality control of SC-EVs, scale-up of production, regulatory control and specific MoA are overcome, SC-EVs therapeutics have the potential to be accelerated into clinical practice.

4.5. Strengths and limitations

To our knowledge, this is the first systematic review and meta-analysis of animal studies evaluating the effectiveness of SC-EVs in treating PBI. Furthermore, we elucidated the therapeutic mechanisms of SC-EVs in immature brain injury from neurons, oligodendrocytes, microglia, astrocytes, vascular epithelial cells and inflammatory factors, and also investigated the factors influencing the effectiveness of SC-EVs. However, our study has several limitations. Firstly, essential information such as randomization and blinding was not reported in the quality assessment of most of the included articles, leading to uncertainty about the risk of bias and affecting the reliability of the results. Secondly, we did not include in vitro potency tests of SC-EVs, only in vivo animal studies, which may not accurately predict the therapeutic potential of SC-EVs (Nguyen et al., 2020). Thirdly, the included studies did not provide exact values, and data had to be extracted from graphs using the online tool WebPlotDigitizer, which might introduce errors and affect the reliability of the results. Finally, the heterogeneity of SC-EVs, the inconsistency of functional assay methods and the limited number of included studies could also affect the conclusions of the analyses.

5. Conclusion

We propose that SC-EVs could potentially improve cognitive and motor functions, as well as brain microstructure. Genetically modified SC-EVs might also help maximize the therapeutic benefits of SC-EVs. This provides a theoretical basis for using cell-free therapies to prevent and/or treat PBI. However, it is essential to investigate and mitigate any potential adverse effects, establish standardized manufacturing criteria, and application protocols for SC-EVs in order to progress cell-

free therapies into clinical practice.

CRediT authorship contribution statement

Mengru Zhong: Methodology, Formal analysis, Investigation. **Simian Cai:** Writing – review & editing. **Xiaolin Guo:** Visualization, Investigation, Writing – original draft, Methodology, Formal analysis. **Tingting Peng:** Methodology, Data curation, Writing – original draft, Formal analysis. **Lu He:** Writing – review & editing, Conceptualization, Supervision. **Kaishou Xu:** Writing – review & editing, Funding acquisition, Investigation, Conceptualization.

Consent for publication

Not applicable.

Ethics

Not applicable.

Funding

The work was supported by the Featured Clinical Technique of Guangzhou (grant number 2023C-TS59), Guangzhou Municipal Science and Technology Project (grant number 2024A03J01274), Plan on enhancing scientific research in Guangzhou Medical University (grant number GMUCR2024–02020), and National Natural Science Foundation of China (grant number 82472598).

Declaration of Generative AI and AI-assisted technologies in the writing process

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.brainresbull.2025.111481.

Data availability

Data will be made available on request.

References

de Abreu, R.C., Fernandes, H., da Costa Martins, P.A., Sahoo, S., Emanueli, C., Ferreira, L., 2020. Native and bioengineered extracellular vesicles for cardiovascular therapeutics. Nat. Rev. Cardiol. 17 (11), 685–697. https://doi.org/10.1038/s41569-020-0389-5.

Alpay Savasan, Z., Kim, S.K., Oh, K.J., Graham, S.F., 2021. Advances in cerebral palsy biomarkers. Adv. Clin. Chem. 100, 139–169. https://doi.org/10.1016/bs.acc.2020.04.006.

Brennan, K., Martin, K., FitzGerald, S.P., O'Sullivan, J., Wu, Y., Blanco, A., Richardson, C., Mc Gee, M.M., 2020. A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid particles in human serum. Sci. Rep. 10 (1), 1039. https://doi.org/10.1038/s41598-020-57497-7.

Busatto, S., Vilanilam, G., Ticer, T., Lin, W.L., Dickson, D.W., Shapiro, S., Bergese, P., Wolfram, J., 2018. Tangential flow filtration for highly efficient concentration of extracellular vesicles from large volumes of fluid. Cells 7 (12). https://doi.org/10.3390/cells7120273.

Campbell, S.J., Carare-Nnadi, R.O., Losey, P.H., Anthony, D.C., 2007. Loss of the atypical inflammatory response in juvenile and aged rats. Neuropathol. Appl. Neurobiol. 33 (1), 108–120. https://doi.org/10.1111/j.1365-2990.2006.00773.x.

Chu, X., Liu, D., Li, T., Ke, H., Xin, D., Wang, S., Cao, Y., Xue, H., Wang, Z., 2020. Hydrogen sulfide-modified extracellular vesicles from mesenchymal stem cells for treatment of hypoxic-ischemic brain injury. J. Control Release 328, 13–27. https://doi.org/10.1016/j.jconrel.2020.08.037.

Drommelschmidt, K., Serdar, M., Bendix, I., Herz, J., Bertling, F., Prager, S., Keller, M., Ludwig, A.K., Duhan, V., Radtke, S., et al., 2017. Mesenchymal stem cell-derived extracellular vesicles ameliorate inflammation-induced preterm brain injury. Brain Behav. Immun. 60, 220–232. https://doi.org/10.1016/j.bbi.2016.11.011.

Gamage, T., Fraser, M., 2021. The role of extracellular vesicles in the developing brain: current perspective and promising source of biomarkers and therapy for perinatal brain injury. Front Neurosci. 15, 744840. https://doi.org/10.3389/fnins.2021.744840.

Gerriets, T., Stolz, E., Walberer, M., Müller, C., Kluge, A., Bachmann, A., Fisher, M., Kaps, M., Bachmann, G., 2004. Noninvasive quantification of brain edema and the space-occupying effect in rat stroke models using magnetic resonance imaging. Stroke 35 (2), 566–571. https://doi.org/10.1161/01.Str.0000113692.38574.57.

Gussenhoven, R., Klein, L., Ophelders, D., Habets, D.H.J., Giebel, B., Kramer, B.W., Schurgers, L.J., Reutelingsperger, C.P.M., Wolfs, T., 2019. Annexin A1 as neuroprotective determinant for blood-brain barrier integrity in neonatal hypoxic-ischemic encephalopathy. J. Clin. Med 8 (2). https://doi.org/10.3390/jcm8020137.

Hamdy, N., Eide, S., Sun, H.S., Feng, Z.P., 2020. Animal models for neonatal brain injury induced by hypoxic ischemic conditions in rodents. Exp. Neurol. 334, 113457. https://doi.org/10.1016/j.expneurol.2020.113457.

Han, J., Yang, S., Hao, X., Zhang, B., Zhang, H., Xin, C., Hao, Y., 2020. Extracellular vesicle-derived microRNA-410 From mesenchymal stem cells protects against neonatal hypoxia-ischemia brain damage through an HDAC1-dependent EGR2/Bcl2 axis. Front Cell Dev. Biol. 8, 579236. https://doi.org/10.3389/fcell.2020.579236.

Hooijmans, C.R., Rovers, M.M., de Vries, R.B., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med Res Method. 14, 43. https://doi.org/10.1186/1471-2288-14-43.

Huang, S., Liu, L., Huang, Y., Fu, C., Peng, T., Yang, X., Zhou, H., Zhao, Y., Xu, Y., Zeng, X., et al., 2023. Potential optimized route for mesenchymal stem cell transplantation in a rat model of cerebral palsy. Exp. Cell Res 430 (2), 113734. https://doi.org/10.1016/j.yexcr.2023.113734.

Jafarinia, M., Alsahebfosoul, F., Salehi, H., Eskandari, N., Ganjalikhani-Hakemi, M., 2020. Mesenchymal stem cell-derived extracellular vesicles: a novel cell-free therapy. Immunol. Invest 49 (7), 758–780. https://doi.org/10.1080/08820139.2020.1712416.

Kaminski, N., Köster, C., Mouloud, Y., Börger, V., Felderhoff-Müser, U., Bendix, I., Giebel, B., Herz, J., 2020. Mesenchymal stromal cell-derived extracellular vesicles reduce neuroinflammation, promote neural cell proliferation and improve oligodendrocyte maturation in neonatal hypoxic-ischemic brain injury. Front Cell Neurosci. 14, 601176. https://doi.org/10.3389/fncel.2020.601176.

Katsha, A.M., Ohkouchi, S., Xin, H., Kanehira, M., Sun, R., Nukiwa, T., Saijo, Y., 2011. Paracrine factors of multipotent stromal cells ameliorate lung injury in an elastase-induced emphysema model. Mol. Ther. 19 (1), 196–203. https://doi.org/10.1038/mt.2010.192.

Keshtkar, S., Azarpira, N., Ghahremani, M.H., 2018. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. Stem Cell Res Ther. 9 (1), 63. https://doi.org/10.1186/s13287-018-0791-7.

Kim, Y.E., Ahn, S.Y., Park, W.S., Sung, D.K., Sung, S.I., Yang, M.S., Chang, Y.S., 2022. Mesenchymal-stem-cell-derived extracellular vesicles attenuate brain injury in escherichia coli meningitis in newborn rats. Life 12 (7). https://doi.org/10.3390/life12071030.

Kimiz-Gebologlu, I., Oncel, S.S., 2022. Exosomes: large-scale production, isolation, drug loading efficiency, and biodistribution and uptake. J. Control Release 347, 533–543. https://doi.org/10.1016/j.jconrel.2022.05.027.

Kordelas, L., Rebmann, V., Ludwig, A.K., Radtke, S., Ruesing, J., Doeppner, T.R., Epple, M., Horn, P.A., Beelen, D.W., Giebel, B., 2014. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. Leukemia 28 (4), 970–973. https://doi.org/10.1038/leu.2014.41.

Labusek, N., Mouloud, Y., Köster, C., Diesterbeck, E., Tertel, T., Wiek, C., Hanenberg, H., Horn, P.A., Felderhoff-Müser, U., Bendix, I., et al., 2023. Extracellular vesicles from immortalized mesenchymal stromal cells protect against neonatal hypoxic-ischemic brain injury. Inflamm. Regen. 43 (1), 24. https://doi.org/10.1186/s41232-023-00274-6.

Laptook, A.R., Shankaran, S., Tyson, J.E., Munoz, B., Bell, E.F., Goldberg, R.N., Parikh, N. A., Ambalavanan, N., Pedroza, C., Pappas, A., et al., 2017. Effect of therapeutic hypothermia initiated after 6 h of age on death or disability among newborns with hypoxic-ischemic encephalopathy: a randomized clinical trial. Jama 318 (16), 1550–1560. https://doi.org/10.1001/jama.2017.14972.

Lawson, A., Snyder, W., Peeples, E.S., 2022. Intranasal administration of extracellular vesicles mitigates apoptosis in a mouse model of neonatal hypoxic-ischemic brain injury. Neonatology 119 (3), 345–353. https://doi.org/10.1159/000522644.

Leavy, A., Jimenez Mateos, E.M., 2020. Perinatal brain injury and inflammation: lessons from experimental murine models. Cells 9 (12). https://doi.org/10.3390/cells9122640.

Li, H., Li, Q., Du, X., Sun, Y., Wang, X., Kroemer, G., Blomgren, K., Zhu, C., 2011. Lithium-mediated long-term neuroprotection in neonatal rat hypoxia-ischemia is associated with antiinflammatory effects and enhanced proliferation and survival of

neural stem/progenitor cells. J. Cereb. Blood Flow. Metab. 31 (10), 2106–2115. https://doi.org/10.1038/jcbfm.2011.75.
Li, P., Lu, X., Hu, J., Dai, M., Yan, J., Tan, H., Yu, P., Chen, X., Zhang, C., 2022. Human amniotic fluid derived-exosomes alleviate hypoxic encephalopathy by enhancing angiogenesis in neonatal mice after hypoxia. Neurosci. Lett. 768, 136361. https://doi.org/10.1016/j.neulet.2021.136361.
Loria, F., Picciotto, S., Adamo, G., Zendrini, A., Raccosta, S., Manno, M., Bergese, P., Liguori, G.L., Bongiovanni, A., Zarovni, N., 2024. A decision-making tool for navigating extracellular vesicle research and product development. J. Extra Vesicles 13 (12), e70021. https://doi.org/10.1002/jev2.70021.
Luo, H., Huang, F., Huang, Z., Huang, H., Liu, C., Feng, Y., Qi, Z., 2022. microRNA-93 packaged in extracellular vesicles from mesenchymal stem cells reduce neonatal hypoxic-ischemic brain injury. Brain Res 1794, 148042. https://doi.org/10.1016/j.brainres.2022.148042.
Malhotra, A., Castillo-Melendez, M., Allison, B.J., Sutherland, A.E., Nitsos, I., Pham, Y., McDonald, C.A., Fahey, M.C., Polglase, G.R., Jenkin, G., et al., 2020. Neurovascular effects of umbilical cord blood-derived stem cells in growth-restricted newborn lambs: UCBCs for perinatal brain injury. Stem Cell Res Ther. 11 (1), 17. https://doi.org/10.1186/s13287-019-1526-0.
Min, W., Wu, Y., Fang, Y., Hong, B., Dai, D., Zhou, Y., Liu, J., Li, Q., 2022. Bone marrow mesenchymal stem cells-derived exosomal microRNA-124-3p attenuates hypoxic-ischemic brain damage through depressing tumor necrosis factor receptor associated factor 6 in newborn rats. Bioengineered 13 (2), 3194–3206. https://doi.org/10.1080/21655979.2021.2016094.
Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6 (7), e1000097. https://doi.org/10.1371/journal.pmed.1000097.
Nguyen, V.V.T., Witwer, K.W., Verhaar, M.C., Strunk, D., van Balkom, B.W.M., 2020. Functional assays to assess the therapeutic potential of extracellular vesicles. J. Extra Vesicles 10 (1), e12033. https://doi.org/10.1002/jev2.12033.
Novak, C.M., Ozen, M., Burd, I., 2018. Perinatal brain injury. Mech. Prev. Outcomes Clin. Perinatol. 45 (2), 357–375. https://doi.org/10.1016/j.clp.2018.01.015.
Ophelders, D.R., Wolfs, T.G., Jellema, R.K., Zwanenburg, A., Andriessen, P., Delhaas, T., Ludwig, A.K., Radtke, S., Peters, V., Janssen, L., et al., 2016. Mesenchymal stromal cell-derived extracellular vesicles protect the fetal brain after hypoxia-ischemia. Stem Cells Transl. Med 5 (6), 754–763. https://doi.org/10.5966/sctm.2015-0197.
Padinharayil, H., Varghese, J., Wilson, C., George, A., 2024. Mesenchymal stem cell-derived exosomes: characteristics and applications in disease pathology and management. Life Sci. 342, 122542. https://doi.org/10.1016/j.lfs.2024.122542.
Pathipati, P., Lecuyer, M., Faustino, J., Strivelli, J., Phinney, D.G., Vexler, Z.S., 2021. Mesenchymal stem cell (MSC)-derived extracellular vesicles protect from neonatal stroke by interacting with microglial cells. Neurotherapeutics 18 (3), 1939–1952. https://doi.org/10.1007/s13311-021-01076-9.
Paton, M.C.B., Wall, D.A., Elwood, N., Chiang, K.Y., Cowie, G., Novak, I., Finch-Edmondson, M., 2022. Safety of allogeneic umbilical cord blood infusions for the treatment of neurological conditions: a systematic review of clinical studies. Cytotherapy 24 (1), 2–9. https://doi.org/10.1016/j.jcyt.2021.07.001.
Paton, M.C.B., Benders, M., Blatch-Williams, R., Dallimore, E., Edwards, A., Elwood, N., Facer, K., Finch-Edmondson, M., Garrity, N., Gordon, A., et al., 2025. Updates on neonatal cell and novel therapeutics: proceedings of the second neonatal cell therapies symposium (2024). Pedia Res. https://doi.org/10.1038/s41390-025-03856-x.
Perlman, J.M., Davis, P., Wyllie, J., Kattwinkel, J., 2010. Therapeutic hypothermia following intrapartum hypoxia-ischemia. an advisory statement from the neonatal task force of the international liaison committee on resuscitation. Resuscitation 81 (11), 1459–1461. https://doi.org/10.1016/j.resuscitation.2010.07.006.
Proietti, J., Boylan, G.B., Walsh, B.H., 2024. Regional variability in therapeutic hypothermia eligibility criteria for neonatal hypoxic-ischemic encephalopathy. Pedia Res 96 (5), 1153–1161. https://doi.org/10.1038/s41390-024-03184-6.
Purcell, E., Nguyen, T., Smith, M., Penny, T., Paton, M.C.B., Zhou, L., Jenkin, G., Miller, S.L., McDonald, C.A., Malhotra, A., 2023. Factors influencing the efficacy of umbilical cord blood-derived cell therapy for perinatal brain injury. Stem Cells Transl. Med 12 (3), 125–139. https://doi.org/10.1093/stcltm/szad006.
Rädler, J., Gupta, D., Zickler, A., Andaloussi, S.E., 2023. Exploiting the biogenesis of extracellular vesicles for bioengineering and therapeutic cargo loading. Mol. Ther. 31 (5), 1231–1250. https://doi.org/10.1016/j.ymthe.2023.02.013.
Riazifar, M., Mohammadi, M.R., Pone, E.J., Yeri, A., Lässer, C., Segaliny, A.I., McIntyre, L.L., Shelke, G.V., Hutchins, E., Hamamoto, A., et al., 2019. Stem cell-derived exosomes as nanotherapeutics for autoimmune and neurodegenerative disorders. ACS Nano 13 (6), 6670–6688. https://doi.org/10.1021/acsnano.9b01004.
Richards, T., Patel, H., Patel, K., Schanne, F., 2023. Endogenous lipid carriers-bench-to-bedside roadblocks in production and drug loading of exosomes. Pharmaceuticals 16 (3). https://doi.org/10.3390/ph16030421.
Sabir, H., Bonifacio, S.L., Gunn, A.J., Thoresen, M., Chalak, L.F., 2021. Unanswered questions regarding therapeutic hypothermia for neonates with neonatal encephalopathy. Semin Fetal Neonatal Med 26 (5), 101257. https://doi.org/10.1016/j.siny.2021.101257.
Semple, B.D., Blomgren, K., Gimlin, K., Ferriero, D.M., Noble-Haeusslein, L.J., 2013. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog. Neurobiol. 106-107, 1–16. https://doi.org/10.1016/j.pneurobio.2013.04.001.
Shaw, O.E.F., Yager, J.Y., 2019. Preventing childhood and lifelong disability: maternal dietary supplementation for perinatal brain injury. Pharm. Res 139, 228–242. https://doi.org/10.1016/j.phrs.2018.08.022.
Shekari, F., Nazari, A., Assar Kashani, S., Hajizadeh-Saffar, E., Lim, R., Baharvand, H., 2021. Pre-clinical investigation of mesenchymal stromal cell-derived extracellular vesicles: a systematic review. Cytotherapy 23 (4), 277–284. https://doi.org/10.1016/j.jcyt.2020.12.009.
Shen, M., Zheng, R., Kan, X., 2022. Neuroprotection of bone marrow-derived mesenchymal stem cell-derived extracellular vesicle-enclosed miR-410 correlates with HDAC4 knockdown in hypoxic-ischemic brain damage. Neurochem. Res 47 (10), 3150–3166. https://doi.org/10.1007/s11064-022-03670-5.
Shu, J., Jiang, L., Wang, R., Wang, M., Peng, Y., Zhu, L., Gao, C., Xia, Z., 2025. Exosomal MiR-653-3p alleviates hypoxic-ischemic brain damage via the TRIM21/p62/Nrf2/CYLD axis. Mol. Neurobiol. 62 (3), 3446–3461. https://doi.org/10.1007/s12035-024-04507-8.
Sisa, C., Kholia, S., Naylor, J., Herrera Sanchez, M.B., Bruno, S., Deregibus, M.C., Camussi, G., Inal, J.M., Lange, S., Hristova, M., 2019. Mesenchymal stromal cell derived extracellular vesicles reduce hypoxia-ischaemia induced perinatal brain injury. Front Physiol. 10, 282. https://doi.org/10.3389/fphys.2019.00282.
Sun, J., Zhang, W., 2024. Huc-MSC-derived exosomal miR-144 alleviates inflammation in LPS-induced preeclampsia-like pregnant rats via the FosB/Flt-1 pathway. Heliyon 10 (2), e24575. https://doi.org/10.1016/j.heliyon.2024.e24575.
Sun, W., Zhao, P., Hu, S., Zhao, Z., Liu, B., Yang, X., Yang, J., Fu, Z., Li, S., Yu, W., 2024. NUFIP1-engineered exosomes derived from hUMSCs regulate apoptosis and neurological injury induced by propofol in newborn rats. Neurotoxicology 102, 81–95. https://doi.org/10.1016/j.neuro.2024.04.002.
Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G.K., et al., 2018. 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. Extra Vesicles 7 (1), 1535750. https://doi.org/10.1080/20013078.2018.1535750.
Thomi, G., Surbek, D., Haesler, V., Joerger-Messerli, M., Schoeberlein, A., 2019b. Exosomes derived from umbilical cord mesenchymal stem cells reduce microglia-mediated neuroinflammation in perinatal brain injury. Stem Cell Res Ther. 10 (1), 105. https://doi.org/10.1186/s13287-019-1207-z.
Thomi, G., Joerger-Messerli, M., Haesler, V., Muri, L., Surbek, D., Schoeberlein, A., 2019a. Intranasally administered exosomes from umbilical cord stem cells have preventive neuroprotective effects and contribute to functional recovery after perinatal brain injury. Cells 8 (8). https://doi.org/10.3390/cells8080855.
van Tilborg, E., de Theije, C.G.M., van Hal, M., Wagenaar, N., de Vries, L.S., Benders, M. J., Rowitch, D.H., Nijboer, C.H., 2018. Origin and dynamics of oligodendrocytes in the developing brain: implications for perinatal white matter injury. Glia 66 (2), 221–238. https://doi.org/10.1002/glia.23256.
Titomanlio, L., Kavelaars, A., Dalous, J., Mani, S., El Ghouzzi, V., Heijnen, C., Baud, O., Gressens, P., 2011. Stem cell therapy for neonatal brain injury: perspectives and challenges. Ann. Neurol. 70 (5), 698–712. https://doi.org/10.1002/ana.22518.
Tscherrig, V., Steinfort, M., Haesler, V., Surbek, D., Schoeberlein, A., Joerger-Messerli, M.S., 2024. All but small: miRNAs from wharton's jelly-mesenchymal stromal cell small extracellular vesicles rescue premature white matter injury after intranasal administration. Cells 13 (6). https://doi.org/10.3390/cells13060543.
Turovsky, E.A., Golovicheva, V.V., Varlamova, E.G., Danilina, T.I., Goryunov, K.V., Shevtsova, Y.A., Pevzner, I.B., Zorova, L.D., Babenko, V.A., Evtushenko, E.A., et al., 2022. Mesenchymal stromal cell-derived extracellular vesicles afford neuroprotection by modulating PI3K/AKT pathway and calcium oscillations. Int J. Biol. Sci. 18 (14), 5345–5368. https://doi.org/10.7150/ijbs.73747.
Wassink, G., Davidson, J.O., Dhillon, S.K., Zhou, K., Bennet, L., Thoresen, M., Gunn, A.J., 2019. Therapeutic hypothermia in neonatal hypoxic-ischemic encephalopathy. Curr. Neurol. Neurosci. Rep. 19 (2), 2. https://doi.org/10.1007/s11910-019-0916-0.
Welsh, J.A., Goberdhan, D.C.I., O'Driscoll, L., Buzas, E.I., Blenkiron, C., Bussolati, B., Cai, H., Di Vizio, D., Driedonks, T.A.P., Erdbrügger, U., et al., 2024. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. J. Extra Vesicles 13 (2), e12404. https://doi.org/10.1002/jev2.12404.
Xiao, S., Lv, Y., Hou, X., Qu, S., 2025. hUC-MSC extracellular vesicles protect against hypoxic-ischemic brain injury by promoting NLRP3 ubiquitination. Biomol. Biomed. 25 (7), 1553–1570. https://doi.org/10.17305/bb.2024.10706.
Xin, D., Li, T., Chu, X., Ke, H., Yu, Z., Cao, L., Bai, X., Liu, D., Wang, Z., 2020. Mesenchymal stromal cell-derived extracellular vesicles modulate microglia/macrophage polarization and protect the brain against hypoxia-ischemic injury in neonatal mice by targeting delivery of miR-21a-5p. Acta Biomater. 113, 597–613. https://doi.org/10.1016/j.actbio.2020.06.037.
Xin, D., Li, T., Chu, X., Ke, H., Liu, D., Wang, Z., 2021. MSCs-extracellular vesicles attenuated neuroinflammation, synapse damage and microglial phagocytosis after hypoxia-ischemia injury by preventing osteopontin expression. Pharm. Res 164, 105322. https://doi.org/10.1016/j.phrs.2020.105322.
Xin, D.Q., Zhao, Y.J., Li, T.T., Ke, H.F., Gai, C.C., Guo, X.F., Chen, W.Q., Liu, D.X., Wang, Z., 2022. The delivery of miR-21a-5p by extracellular vesicles induces microglial polarization via the STAT3 pathway following hypoxia-ischemia in neonatal mice. Neural Regen. Res 17 (10), 2238–2246. https://doi.org/10.4103/1673-5374.336871.
Xylaki, M., Chopra, A., Weber, S., Bartl, M., Outeiro, T.F., Mollenhauer, B., 2023. Extracellular vesicles for the diagnosis of parkinson's disease: systematic review and meta-analysis. Mov. Disord. 38 (9), 1585–1597. https://doi.org/10.1002/mds.29497.
Yang, Z., Liang, Z., Rao, J., Lin, F., Lin, Y., Xu, X., Wang, C., Chen, C., 2023. Mesenchymal stem cell-derived extracellular vesicles therapy in traumatic central nervous system diseases: a systematic review and meta-analysis. Neural Regen. Res 18 (11), 2406–2412. https://doi.org/10.4103/1673-5374.371376.

Yates, N., Gunn, A.J., Bennet, L., Dhillon, S.K., Davidson, J.O., 2021. Preventing brain injury in the preterm infant-current controversies and potential therapies. Int J. Mol. Sci. 22 (4). https://doi.org/10.3390/ijms22041671.

Yıldız, E.P., Ekici, B., Tatlı, B., 2017. Neonatal hypoxic ischemic encephalopathy: an update on disease pathogenesis and treatment. Expert Rev. Neurother. 17 (5), 449–459. https://doi.org/10.1080/14737175.2017.1259567.

Zhang, M., Zang, X., Wang, M., Li, Z., Qiao, M., Hu, H., Chen, D., 2019. Exosome-based nanocarriers as bio-inspired and versatile vehicles for drug delivery: recent advances and challenges. J. Mater. Chem. B 7 (15), 2421–2433. https://doi.org/10.1039/c9tb00170k.

Zhang, X., Zhang, H., Gu, J., Zhang, J., Shi, H., Qian, H., Wang, D., Xu, W., Pan, J., Santos, H.A., 2021. Engineered extracellular vesicles for cancer therapy. Adv. Mater. 33 (14), e2005709. https://doi.org/10.1002/adma.202005709.

Zhao, J., Deng, H., Xun, C., Chen, C., Hu, Z., Ge, L., Jiang, Z., 2023. Therapeutic potential of stem cell extracellular vesicles for ischemic stroke in preclinical rodent models: a meta-analysis. Stem Cell Res Ther. 14 (1), 62. https://doi.org/10.1186/s13287-023-03270-2.

Zhu, C., Wang, X., Xu, F., Bahr, B.A., Shibata, M., Uchiyama, Y., Hagberg, H., Blomgren, K., 2005. The influence of age on apoptotic and other mechanisms of cell death after cerebral hypoxia-ischemia. Cell Death Differ. 12 (2), 162–176. https://doi.org/10.1038/sj.cdd.4401545.