



Embryonic stem cell-derived neural stem cell transplantation in experimental ischemic stroke: A systematic review and meta-analysis

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Keywords

Neural Stem Cells; Embryonic Stem Cells; Stroke Rehabilitation; Neurodegenerative Diseases; Cerebral Infarction

Abstract

Background: Stem cell therapy holds promise for neural repair after ischemic injury. This systematic review and meta-analysis evaluated the efficacy of embryonic stem cell-derived neural stem/progenitor cells (ESC-NSCs) in experimental ischemic stroke.

Methods: Preclinical animal studies were identified through MEDLINE, Embase, Scopus, and Web of Science by February 2024. Screening, data extraction, and risk-of-bias assessment (SYRCLE tool) were performed independently by two reviewers.

Results: Twenty-three studies met inclusion criteria. Pooled analyses indicated that ESC-NSC transplantation improved locomotion [standardized mean difference (SMD) = 0.57; 95% confidence interval (CI): 0.34 to 0.80; $P < 0.001$] and neurological status assessed by the cylinder test (SMD = 0.69; 95% CI: 0.29 to 1.08; $P = 0.001$), while reducing infarct volume (SMD = -0.26; 95% CI: -0.50 to -0.02; $P = 0.030$) and astrogliosis [glial fibrillary acidic protein (GFAP)⁺ cells; SMD = -3.02; 95% CI: -3.87 to -2.18; $P < 0.001$].

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No significant effect was observed on inflammation [ionizing calcium-binding adaptor molecule 1 (Iba-1)⁺ cells; SMD = -3.69; 95% CI: -10.03 to 2.64; $P = 0.254$]. Subgroup analyses showed greater locomotor improvement when ESC-NSCs were administered 24-72 hours after stroke, in both neonatal and adult models. Most included studies had an unclear risk of bias in randomization and blinding domains. According to Grading of Recommendations Assessment, Development, and Evaluation (GRADE), the certainty of evidence was low to moderate for the main outcomes.

Conclusion: ESC-NSC transplantation may enhance locomotor and neurological recovery and reduce tissue damage in experimental ischemic stroke, particularly when administered in the early post-ischemic phase. However, due to methodological heterogeneity, small sample sizes, and low-to-moderate certainty of evidence, these findings should be interpreted with caution.

Introduction

Stroke is one of the main causes of death and long-term disability worldwide, with an incidence of 84.2 deaths per 100000 people each year.¹ The majority of strokes are ischemic in origin, where functional recovery largely depends on the timely rescue of the ischemic penumbra. Traditional early reperfusion techniques such as revascularization and thrombolysis have been the primary treatments aimed at saving this vital area.² However, due to their limited therapeutic window, only 5% of patients with stroke benefit from these interventions.³

Given these limitations, there has been significant interest in exploring alternative therapeutic strategies that offer a longer window for intervention. Stem cell therapies, derived from two primary sources – embryonic stem cells (ESCs) and adult (somatic) stem cells – have shown promise due to their potential for neuroregeneration and functional recovery.⁴ These therapies operate through multiple regenerative mechanisms such as angiogenesis, neurogenesis, synaptic plasticity, cell replacement, and immune modulation, leading to both structural and functional regeneration of brain tissues.⁵

ESCs, which can differentiate into various lineages, have been shown to produce neural progenitor cells under specific protocols. These progenitor cells can integrate into the brain, forming functional neurons and potentially reducing cerebrovascular injury and inflammation.⁶ This approach has demonstrated

effectiveness in animal models of stroke,^{7,8} but several challenges remain, including identifying the optimal cell source for promoting neural repair.

This systematic review and meta-analysis focuses on the effectiveness of ESC-derived neural stem/progenitor cells (ESC-NSCs) in treating ischemic stroke. ESC-NSCs, which are capable of generating specific neural cell types, offer a promising avenue for transplantation therapies. They not only replace damaged neurons but also produce various trophic, plasticity, and immunomodulatory factors that can aid in stroke recovery.^{9,10}

Despite encouraging preclinical findings,^{11,12} clinical evidence for ESC-NSCs in ischemic stroke remains limited. To date, no comprehensive synthesis has quantified their efficacy across experimental models. Therefore, this study systematically reviews and meta-analyzes preclinical data to determine whether ESC-NSC transplantation improves functional recovery and reduces infarct burden after ischemic stroke, and how these effects vary by timing of administration, animal model, age (neonatal vs. adult), and other methodological factors.

Materials and Methods

Study design and search strategy: This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines.¹³ Although the study protocol has not been previously registered or published, our approach aligns with rigorous standards and predefined selection criteria.

The search strategy involved selecting relevant keywords from existing literature, exploring medical subject headings (MeSH) and Embase subject headings (Emtree) terms, and consulting field experts. Online databases, including MEDLINE (via PubMed), Embase, Scopus, and Web of Science, were searched until February 6, 2024, using the identified keywords, standard tags, and Boolean operators. Additionally, manual searches were conducted on Google, Google Scholar, and Semantic Scholar for preprints and other potentially relevant records not indexed in the systematic search. Reference lists of the included studies, as well as articles citing these studies, were reviewed to identify additional relevant studies. No restrictions were imposed on language or publication date. Supplementary material provides the search queries used across all explored databases.

Screening of obtained records, inclusion of

potentially relevant studies, full-text screenings, and data extraction were all done independently by two reviewers. Any discrepancies were resolved by discussion and if needed with the opinion of third senior reviewer. Non-English articles were intended to be translated using online services such as Doc Translator, and then have them reviewed by an expert translator fluent in both the original language and English to ensure accuracy.

Selection criteria: The definition of Population, Intervention, Comparison, and Outcome (PICO) in the present study was as follows: The population (P) of the study were animals (rats or mice) with ischemic stroke, intervention (I) was the transplantation of ESC-NSC, the comparison (C) was made with a control group (ischemic stroke with no treatment), and the study outcomes (O) were the motor and neurological recovery, the size of the infarct area, neuroprotection, astrogliosis, and inflammation. Exclusion criteria included studies without a control group, studies that administered a conditioned medium instead of ESC-NSC, Studies featuring combination therapies, excluding those utilizing ESC-NSC monotherapy as a standalone treatment arm, studies that failed to report the desired outcome, in-vitro studies, duplicate studies, abstracts with insufficient data, case reports, and reviews. Studies with administration of other types of stem cell or neural stem cells (NSCs) derived from sources other than embryo were also excluded.

Study selection process and data extraction: The results of the systematic search were imported into Endnote X9.0 software, and duplicate reports were removed. During the initial screening, two researchers worked independently to evaluate the titles and abstracts of the identified articles and determine potentially relevant studies. Subsequently, the full texts of these selected articles underwent a thorough review, during which the inclusion and exclusion criteria were applied. Articles that met these criteria were included in the study.

The obtained data included the bibliographic information of the articles, the sample size and animals' characteristics, the stroke induction model, duration of ischemia, number of transplanted cells, administration method, injury-to-treatment interval, and injury-to-outcome assessment interval (follow-up). If multiple follow-ups were reported for the outcome measurement, the data from the longest follow-up were included in the meta-analysis.

For each eligible study, when numerical data were available only in graphical form, two reviewers independently extracted values using Plot Digitizer software. The mean of their measurements was used in the analysis. If discrepancies exceeded 5%, the extraction was repeated to ensure accuracy. Any remaining disagreements were resolved through discussion with a third reviewer.

Outcomes: In our study, we assessed a comprehensive array of locomotion and neurological status outcomes to evaluate the impact of ESC-NSC transplantation on motor and sensory function. Neurological status was evaluated and stratified using three tests: the original or modified version of neurological severity scale (NSS/mNSS), cylinder test, and adhesive removal test. Astrogliosis, neuroprotection, and inflammation were predefined based on markers assessed in at least three studies, resulting in the inclusion of glial fibrillary acidic protein (GFAP)⁺, neuronal nuclei (NeuN)⁺, and ionizing calcium-binding adaptor molecule 1 (Iba-1)⁺ cell counts, respectively.

Risk of bias assessment and certainty of evidence: The risk of bias of the included studies were evaluated by two independent reviewers using SYRCLE's risk of bias assessment tool.¹⁴ For evaluation of the certainty of evidence for each outcome, we used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE).¹⁵ Any conflicts or disagreements were resolved through discussions with a third researcher.

Statistical analyses: The statistical analyses for this study were conducted using Stata software (version 17.0, Stata Corporation, College Station, TX, USA). Analyses were stratified based on outcomes. For locomotion, when a study reported more than one outcome, the most frequently reported outcome was included in the analysis. For neurological status, analyses were stratified by test, and each test was analyzed separately.

Because locomotor and histological outcomes were reported using different behavioral tests and measurement scales across studies, effect sizes were calculated as standardized mean differences (SMDs) with 95% confidence intervals (CIs). The SMD standardizes the difference between treatment and control means by the pooled standard deviation (SD), allowing results from heterogeneous scales to be combined in a unified meta-analysis. For each analysis, we computed

SMD using Hedge's *g* method, accompanied by a 95% CI. These SMD values were then aggregated to determine an overall effect size. A meta-analysis was conducted only when data were available from at least three distinct studies. The random effects model was chosen, primarily due to the methodological heterogeneity observed among the included studies. This model accounts for variations among the included studies, thereby providing a more conservative estimate of the overall effect size in the presence of such heterogeneity and minimizing the type 1 error.

Forest plots were examined to identify potential outlier studies, particularly those with extremely high absolute effect sizes or effect directions that differed from the rest. To further detect potential outlier studies, a Galbraith plot was employed. This plot provides a visual representation of study precision and effect size, making it a widely used tool for identifying studies that contribute disproportionately to heterogeneity in meta-analyses. The method has been recommended in meta-analytic research for detecting potential outlier studies.¹⁶⁻¹⁸

For each identified outlier, a leave-one-out sensitivity analysis was performed to assess its influence on the overall effect estimate. If an outlier significantly altered the pooled effect, it was determined influential and excluded from the final meta-analysis.

To assess statistical heterogeneity, we visually inspected forest plots and employed the I^2 and chi-square tests. Furthermore, when data from at least ten experiments related to a specific outcome were available, subgroup analyses were conducted to evaluate the efficacy of cell transplantation across different subgroups.

Between-study heterogeneity was explored using predefined subgroup analyses based on animal species, stroke model, occlusion time, age, interval from stroke to transplantation, graft type, number of transplanted cells, and use of immunosuppression to identify potential sources of variability. To assess the robustness of the findings, sensitivity analyses were also performed, excluding studies that used a hypoxic chamber for the induction of hypoxic-ischemic injury. Additionally, for each outcome, publication bias was assessed using a funnel plot and the modified Egger's test suggested by Doleman et al.¹⁹

Results

Study selection: The systematic search yielded

5970 records. Upon removal of duplicates, 4454 records underwent title and abstract screening, with 103 articles being selected for full-text review. Ultimately, data from 23 articles were included in the meta-analysis.^{6-8,11,12,20-37} Additional searches, including forward and backward citation tracking of included studies and targeted gray literature searches on Google and Google Scholar yielded no further eligible articles (Figure 1). All relevant texts were retrieved in English and did not require translation.

Study characteristics: The included studies comprised 12 conducted on rats and 11 on mice. The predominant model for cerebral ischemia was the middle cerebral artery occlusion (MCAO). Regarding the method of occlusion, eight studies utilized a permanent model, 15 employed a transient model, and one involved the injection of endothelin-1 (ET-1) into the sensorimotor cortex. The duration of vascular occlusion in studies employing the transient model ranged from 7 to 180 minutes. Four studies focused on neonatal rodents aged between 2 to 10 days, where ligation of the common carotid artery (CCA) was followed by exposure to a hypoxic chamber for 15 to 90 minutes. The timing for the administration of ESC-NSC post-ischemia varied from 1 day to 3 weeks, with all studies administering the cells intracerebrally. Transplantation methods included allografts in five studies and xenografts in 18 studies, with the number of transplanted cells ranging from 2.5×10^4 to 6×10^6 cells. Follow-up durations extended from 2 days to 36 weeks. Outcome assessments included locomotion (13 studies), neurological status (15 studies), infarct size (12 studies), gliosis (4 studies), neural differentiation (4 studies), and inflammation (3 studies) (Table 1).

Risk of bias in studies: The allocation concealment was appropriately conducted in nine of the included studies. Baseline characteristics were similar between experimental groups in all of the studies. None of the studies clearly disclosed sequence generation, nor was there documentation regarding the randomization process. Caregiver blinding was not reported in any of the studies, whereas outcome assessor blinding was specified in 10 studies. Except for 6 studies, it was unclear whether a random selection process was used for outcome assessment in the remaining studies. There appeared to be no incomplete outcome data, selective outcome reporting, or other factors that could potentially cause bias (Table 2).

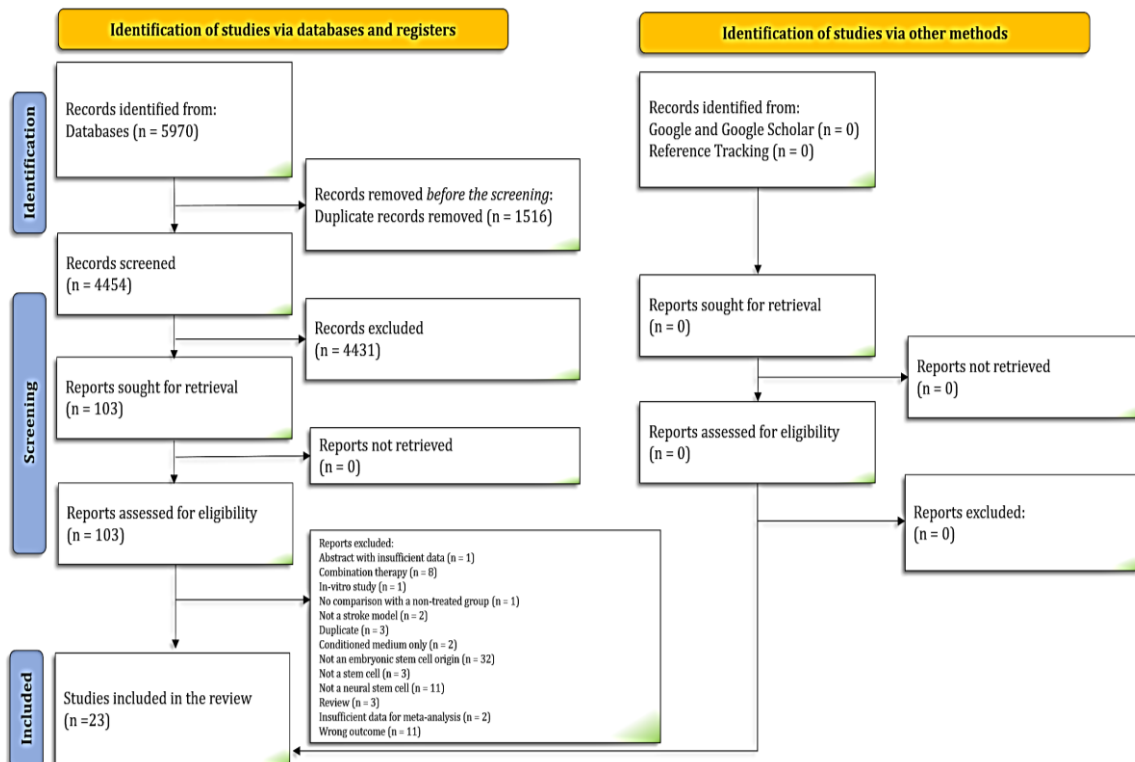


Figure 1. The PRISMA flow diagram depicting the flow of the study selection process through the different phases of the present systematic review

Meta-analysis

Locomotion recovery: Locomotion was assessed using the rotarod, elevated body swing, beam balance, foot-fault, horizontal ladder, and staircase tests. Data from 14 experiments were analyzed.

Pooled data analysis indicated that the administration of ESC-NSC at the injury site significantly improved the locomotion of animals (SMD = 0.57; 95% CI: 0.34 to 0.80; $P < 0.001$; $I^2 = 0.00\%$) with no statistical heterogeneity (Figure 2).

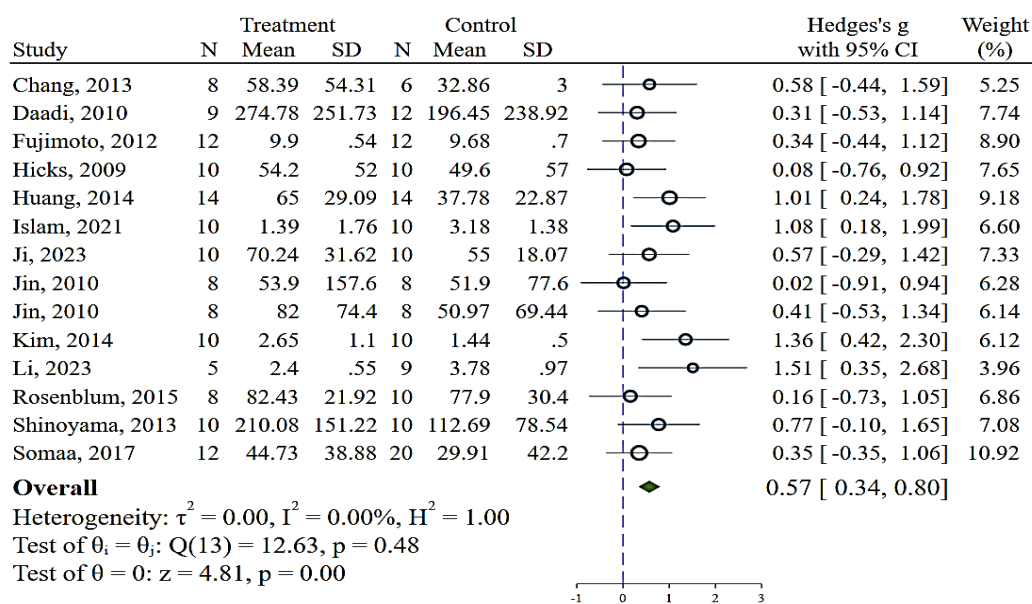


Figure 2. Forest plot for the efficacy of embryonic stem cell-derived neural stem/progenitor cells (ESC-NSCs) on locomotion recovery following ischemic stroke (CI: Confidence interval; SD: Standard deviation)

Table 1. Characteristics of included studies

Study (first author)	Gender/species/strain/weight (g)	Model of stroke/occlusion time (minute)	Stroke to treatment (hour)/route of administration/type of graft/donor of cell/number of transplanted cells	Immunosuppressive	Outcome	Follow-up duration (day)	Number of control/number of treated
Arab ¹¹	Male/rat/Wistar/260-300	CCA and ECA ligation/60	24/IC/xenograft/human/ 1×10^5	No	Sensory-motor	7	8/8
Chang ²⁰	Male/rat/Sprague-Dawley/270-300	MCAO/90	168/IC/xenograft/human/ 2×10^5	Cyclosporine	Motor, sensory-motor	56	6/8
Daadi ²¹	Male/rat/Sprague-Dawley/275-310	MCAO/90	168/IC/xenograft/human/ 1×10^5	Cyclosporine	Sensory-motor	56	7/10
Daadi ²²	NR/rat/Sprague-Dawley/NR	LCA permanent ligation/90	24/IC/xenograft/human/ 3×10^5	No	Motor	30	12/9
Daadi ²³	Male/rat/Sprague-Dawley/275-310	MCAO/90	168/IC/xenograft/human/ 5×10^4 , 5×10^5 , and 1×10^6	Cyclosporine	Motor, sensory-motor, infarct size	70	24/24
Drury-Stewart ²⁴	Male/mice/C57BL/6/NR	MCA distal ligation/7	168/IC/xenograft/human/ 2×10^5	No	Sensory-motor	28	17/18
Fujimoto ²⁵	Male/mice/C57BL/6/25-30	MCAO/45	168/IC/allograft/mice/ 2×10^5	No	Motor, sensory-motor	56	12/12
Guan ²⁶	Male/rat/Wistar/NR	MCAO/120	48/IC/allograft/mice/ 5×10^5	Cyclosporine	Sensory-motor	28	10/10
Hamblin ¹²	Male/mice/C57BL/6/NR	MCAO/60	24/IC/xenograft/human/ 1×10^5	No	Infarct size	2	7/9
Hayashi ²⁷	NR/mice/C57BL/6/20-25	MCAO/30	24/IC/xenograft/monkey/ 2.5×10^5	Cyclosporine	Infarct size	28	5/5
Hicks ²⁸	Male/rat/Wistar/275-300	MCAO + carotid ligation/permanent	168/IC/xenograft/human/ 8×10^5	Cyclosporine	Motor, sensory-motor, infarct size	60	
Huang ⁶	Male/mice/C57BL/6/24-26	MCAO/60	24/IC/xenograft/human/ 1×10^5	No	Motor, sensory-motor, infarct size	24	14/14
Islam ²⁹	Male/mice/Fox Chase SCID/Beige/NR	Injection to cortex/NR	96/IC/xenograft/human/ 1×10^5	No	Motor, inflammation	20	10/10
Ji ⁷	NR/mice/SCID/NR	LCCA ligation/permanent	336/IC/xenograft/human/NR	No	Motor	182	10/10
Jin ³⁰	Male/rat/Sprague-Dawley/280-310	MCAO/permanent	504/IC/xenograft/human/ 6×10^6	No	Infarct size	35	
Jin ³¹	Male/rat/Fisher 344/280-310	MCA distal occlusion/permanent	504/IC/xenograft/human/ 1.2×10^6	Cyclosporine	Motor, sensory-motor, infarct size	56	16/16
Kim ³²	Male/rat/Sprague-Dawley/250-300	pMCAO/permanent	48/IC/xenograft/human/ 5×10^5	Cyclosporine	Motor, sensory-motor, infarct size	24	10/10
Li ⁸	Male/rat/Sprague-Dawley/280-320	pMCAO/permanent	48/IC/xenograft/human/ 3×10^5	No	Motor, sensory-motor, infarct size, neural differentiation, inflammation	28	12/10

Table 1. Characteristics of included studies (continue)

Study	Gender/species/strain/weight (g)	Model of stroke/occlusion time (minute)	Stroke to treatment (hour)/route of administration/type of graft/donor of cell/number of transplanted cells	Immunosuppressive	Outcome	Follow-up duration (day)	Number of control/number of treated
Patkar ³³	Male/mice/C57BL/6/25-30	MCAO/45	48/IC/allograft/mice/ 2.5×10^4	No	Sensory-motor, infarct size, gliosis, inflammation	28	4/6
Rosenblum ³⁴	Male/mice/NR/NR	LCA occlusion/15	72/IC/xenograft/human/ 5×10^5	No	Motor	28	10/8
Shabani ³⁵	Male/mice/BALB/c/20-22	Photothrombotic/10	168/IC/allograft/mice/ 2.5×10^5	No	Motor, infarct size, neural differentiation, gliosis	28	3/3
Shinoyama ³⁶	NR/mice/ICR/NR	RCCA ligation/120	48/IC/allograft/mice/ 2×10^5	No	Motor	21	10/10
Somaa ³⁷	NR/rat/Athymic CBH/NR	Injection to cortex/180	504/IC/xenograft/human/ 1×10^5	No	Motor	252	20/12

CCA: Common carotid artery; ECA: External carotid artery; IC: Intracerebral injections; MCA: Middle cerebral artery; MCAO: Middle cerebral artery occlusion; pMACO: Permanent middle cerebral artery occlusion; LCA: Left coronary artery; LCCA: Left common carotid artery; RCCA: Right common carotid artery; SCID: Severe combined immunodeficiency; NR: Not reported

Table 2. Risk of bias assessment of the included studies based on the SYRCLE tool

Study (first author)	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding trial caregivers	Random outcome assessment	Blinding outcome assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Arab ¹¹	U	L	L	U	U	U	U	L	L	L
Chang ²⁰	U	L	U	U	U	U	U	L	L	L
Daadi ²¹	U	L	U	U	U	U	L	L	L	L
Daadi ²²	U	L	L	U	U	U	U	L	L	L
Daadi ²³	U	L	U	U	U	U	L	L	L	L
Drury-Stewart ²⁴	U	L	U	U	U	U	L	L	L	L
Fujimoto ²⁵	U	L	U	U	U	U	U	L	L	L
Guan ²⁶	U	L	U	U	U	U	L	L	L	L
Hamblin ¹²	U	L	U	U	U	U	U	L	L	L
Hayashi ²⁷	U	L	U	U	U	L	U	L	L	L
Hicks ²⁸	U	L	U	U	U	L	U	L	L	L
Huang ⁶	U	L	U	U	U	U	U	L	L	L
Islam ²⁹	U	L	U	U	U	U	U	L	L	L
Ji ⁷	U	L	L	U	U	L	U	L	L	L
Jin ³⁰	U	L	U	U	U	U	L	L	L	L
Jin ³¹	U	L	U	U	U	U	L	L	L	L
Kim ³²	U	L	L	U	U	U	L	L	L	L
Li ⁸	U	L	L	U	U	U	L	L	L	L
Patkar ³³	U	L	L	U	L	L	L	L	L	L
Rosenblum ³⁴	U	L	L	U	U	U	L	L	L	L
Shabani ³⁵	U	L	L	U	U	L	U	L	L	L
Shinoyama ³⁶	U	L	L	U	U	L	U	L	L	L
Somaa ³⁷	U	L	U	U	U	U	U	L	L	L

L: Low risk of bias; U: Unclear risk of bias; H: High risk of bias

The improvement in locomotion was observed in both rats (SMD = 0.50; 95% CI: 0.17 to 0.82; $P = 0.003$) and mice (SMD = 0.66; 95% CI: 0.31 to 1.00; $P < 0.001$). The enhancements were evident regardless of whether ischemia was transient (7 to 180 minutes) (SMD = 0.49; 95% CI: 0.13-0.86; $P = 0.008$) or permanent (SMD = 0.57; 95% CI: 0.23 to 0.91; $P = 0.001$). Improvements were noted in both adult (SMD = 0.62; 95% CI: 0.32 to 0.92; $P < 0.001$) and neonatal animals exposed to hypoxic conditions, simulating hypoxic-ischemic encephalopathy (HIE) (SMD = 0.45; 95% CI: 0.02 to 0.88; $P = 0.040$). The treatment was effective during follow-up durations of ≤ 4 weeks (SMD = 0.94; 95% CI: 0.57 to 1.31; $P < 0.001$) and > 4 weeks (SMD = 0.33; 95% CI: 0.03 to 0.63; $P = 0.033$). Interestingly, ESC-NSC transplantation improved locomotion only in MCAO models (SMD = 0.71; 95% CI: 0.32 to 1.09; $P < 0.001$), not in CCA occlusion (CCAO) models (SMD = 0.37; 95% CI: -0.01 to 0.76; $P = 0.056$). The treatment was effective only in the acute phase (within 72 hours

post-injury) (SMD = 0.84; 95% CI: 0.49-1.19; $P < 0.001$) and without the administration of cyclosporine for immunosuppression (SMD = 0.62; 95% CI: 0.27 to 0.97; $P = 0.001$) (Table 3).

Neurological status recovery: Due to methodological heterogeneity among tests assessing neurological status recovery, the analysis was stratified by test type into three groups: the cylinder test, the NSS/mNSS, and the adhesive removal test.

Pooled data from 7 experiments indicated that ESC-NSC transplantation significantly improved spontaneous forelimb movements, as evaluated by the cylinder test (SMD = 0.69; 95% CI: 0.29 to 1.08; $P = 0.001$; $I^2 = 16.44\%$). On the other hand, the results showed no evidence that ESC-NSC treatment improved the mean time to remove adhesive from the forepaws (adhesive removal test: SMD = 1.59; 95% CI: -0.81 to 3.99; $P = 0.194$; $I^2 = 96.66\%$) or the neurological deficit (NSS/mNSS: SMD = 1.36; 95% CI: -0.0002 to 2.7300; $P = 0.050$; $I^2 = 86.01\%$) in stroke-induced animals (Figure 3).

Table 3. Subgroup analysis for the effect of embryonic stem cell-derived neural stem/progenitor cells (ESC-NSCs) on locomotion recovery following stroke

Subgroup	No. experiment	SMD (95% CI)	P	Heterogeneity [% (P)]
Animal species				
Rat	8	0.50 (0.17, 0.82)	0.003	5.3 (0.288)
Mice	6	0.66 (0.31, 1.00)	< 0.001	77.1 (0.004)
Time of occlusion				
7 to 180 minutes	5	0.49 (0.13, 0.86)	0.008	0.0 (0.623)
Permanent	8	0.57 (0.23, 0.91)	0.001	10.7 (0.283)
Age				
Neonate	4	0.45 (0.02, 0.88)	0.040	0.0 (0.771)
Adult	10	0.62 (0.32, 0.92)	< 0.001	14.6 (0.269)
Model of stroke induction				
MCAO	7	0.71 (0.32, 1.09)	< 0.001	19.5 (0.259)
CCAO	5	0.37 (-0.01, 0.76)	0.056	0.0 (0.787)
Interval of stroke to treatment (hour)				
24-72	7	0.84 (0.49, 1.19)	< 0.001	7.3 (0.344)
96-168	3	0.31 (-0.19, 0.80)	0.228	0.0 (0.758)
336-504	4	0.35 (-0.07, 0.76)	0.106	0.0 (0.859)
Type of graft				
Allograft	2	Insufficient data	Insufficient data	Insufficient data
Xenograft	12	0.58 (0.32, 0.83)	< 0.001	2.4 (0.358)
Number of transplanted cells				
$< 5 \times 10^5$	8	0.67 (0.37, 0.97)	< 0.001	0.0 (0.556)
$\geq 5 \times 10^5$	5	0.39 (-0.08, 0.86)	0.105	25.3 (0.242)
Administration of immunosuppressive				
Not administered	6	0.62 (0.27, 0.97)	0.001	0.0 (0.365)
Cyclosporine	5	0.47 (-0.01, 0.95)	0.054	24.3 (0.264)
Follow-up duration (week)				
≤ 4	6	0.94 (0.57, 1.31)	< 0.001	0.0 (0.425)
> 4	8	0.33 (0.03, 0.63)	0.033	0.0 (0.998)

CI: Confidence interval; MCAO: Middle cerebral artery occlusion; CCAO: Common carotid artery occlusion; SMD: Standardized mean difference

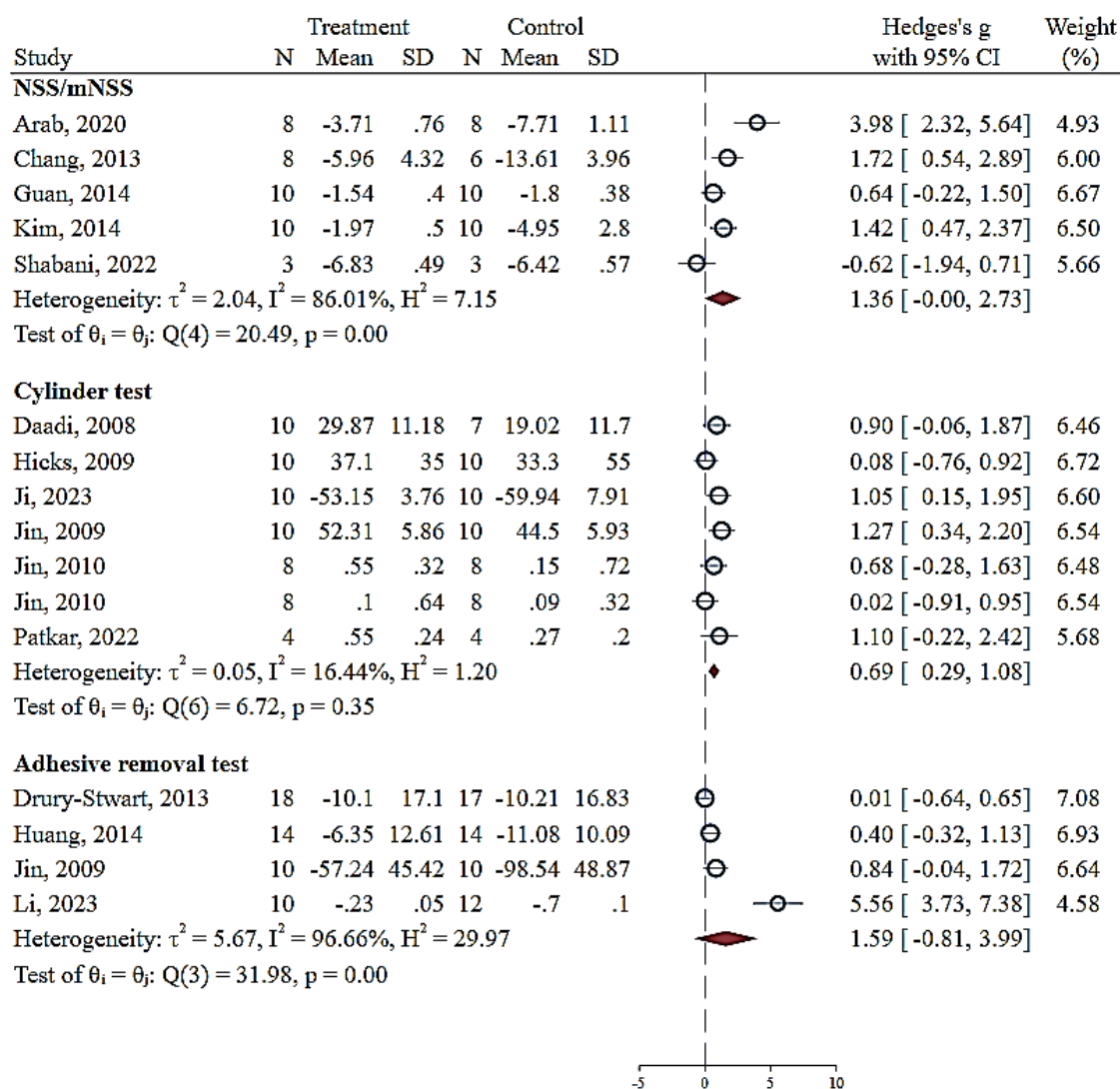


Figure 3. Forest plot for the efficacy of embryonic stem cell-derived neural stem/progenitor cells (ESC-NSCs) on neurological status following ischemic stroke (CI: Confidence interval; SD: Standard deviation)

Subgroup analysis was not applicable due to limited data in this part of the study.

Infarct size: The comparison of infarct sizes between treated and untreated animals was conducted using histological examination or magnetic resonance imaging (MRI). This section includes data from 12 studies with 18 separate experiments in the meta-analysis. Intracerebral administration of ESC-NSC significantly reduced infarct size after the longest follow-up period in each study (SMD = -0.26; 95% CI: -0.50 to -0.02; $P = 0.030$; $I^2 = 0.00\%$), indicating no statistical heterogeneity (Figure 4).

The reduction in infarct size was evident in mice (SMD = -1.05; 95% CI: -1.94 to -0.16; $P = 0.021$) but not in rats (SMD = -0.12; 95% CI: -0.38 to 0.15; $P = 0.391$), and only in cases of

transient ischemia (SMD = -0.32; 95% CI: -0.630 to -0.002; $P = 0.049$). This reduction was observed only when animals were treated in the acute phase (within 72 hours post-injury) (SMD = -0.73; 95% CI: -1.40 to -0.06). When animals were followed for more than 4 weeks, the ameliorative effect of ESC-NSC on infarct size became non-significant (SMD = -0.01; 95% CI: -0.33 to 0.31; $P = 0.949$). Due to the reduced number of studies, the effect size became insignificant in all subgroups based on the number of transplanted cells and the administration of immunosuppressive drugs (Table 4).

Astrocytosis: Pooled data showed that the administration of ESC-NSCs could alter the levels of astrocytic gliosis in the ischemic area compared to the untreated stroke group (Figure 5).

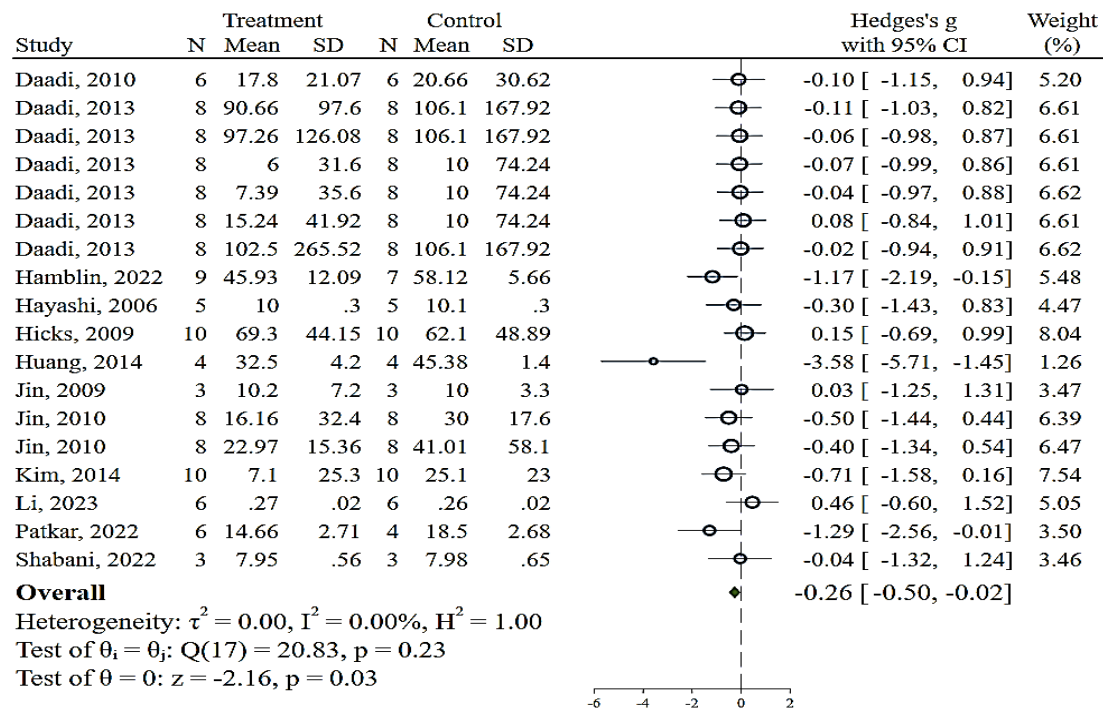


Figure 4. Forest plot for the efficacy of embryonic stem cell-derived neural stem/progenitor cells (ESC-NSCs) on infarct size following ischemic stroke (CI: Confidence interval; SD: Standard deviation)

According to our findings, the administration of ESC-NSCs can significantly reduce the number of recruited astrocytes (GFAP⁺ cells) in the periphery and inside the necrotic area compared to the control

stroke group (SMD = -3.02; 95% CI: -3.87 to -2.18; $P < 0.001$; $I^2 = 0.00$), indicating no statistical heterogeneity. Subgroup analysis was not applicable due to limited data in this part of the study.

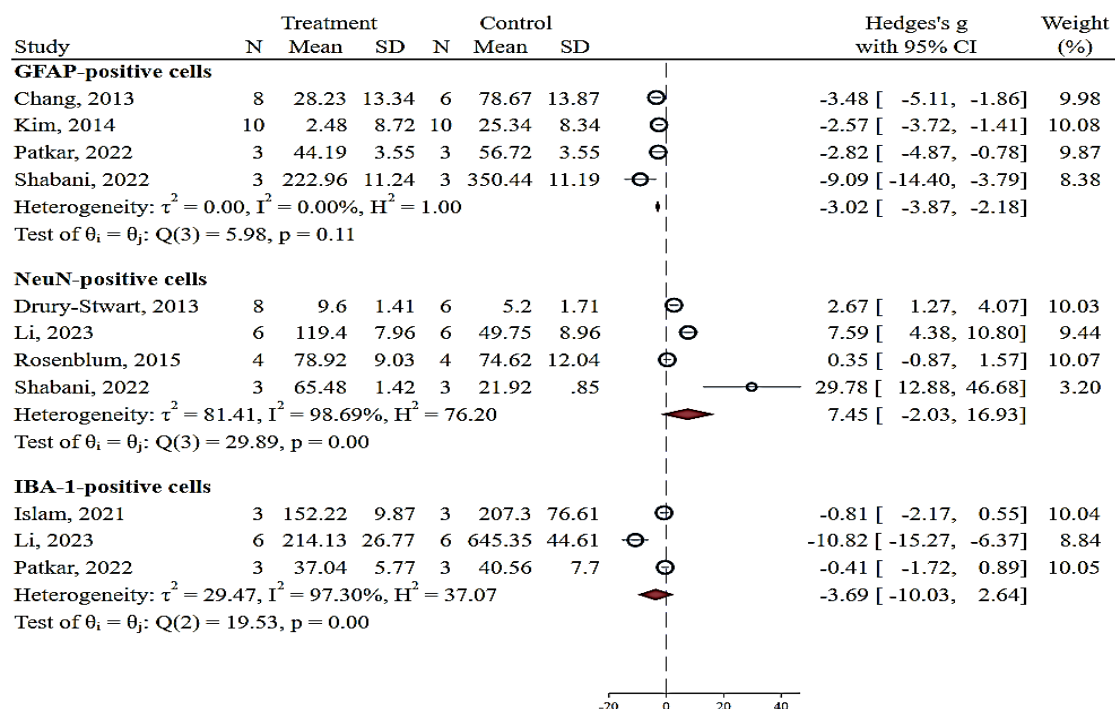


Figure 5. Forest plot for the efficacy of embryonic stem cell-derived neural stem/progenitor cells (ESC-NSCs) on astrocytosis, neuroprotection, and inflammation following ischemic stroke (CI: Confidence interval; SD: Standard deviation)

Table 4. Subgroup analysis for the effect of embryonic stem cell-derived neural stem/progenitor cells (ESC-NSCs) on infarct size following stroke

Subgroup	No. experiment	SMD (95% CI)	P	Heterogeneity [% (P)]
Animal species				
Rat	13	-0.120 (-0.380, 0.150)	0.391	0.0 (0.968)
Mice	5	-1.050 (-1.940, -0.160)	0.021	57.8 (0.049)
Time of occlusion				
7 to 180 minutes	11	-0.320 (-0.630, -0.002)	0.049	0.0 (0.090)
Permanent	7	-0.190 (-0.560, 0.180)	0.306	0.0 (0.647)
Age				
Neonate	1	Insufficient data		
Adult	17	-0.270 (-0.520, -0.030)	0.029	0.0 (0.189)
Model of stroke induction				
MCAO	15	-0.320 (-0.580, -0.060)	0.016	0.0 (0.417)
CCAO	2	Insufficient data		
Interval of stroke to treatment (hour)				
24-72	7	-0.730 (-1.400, -0.060)	0.033	58.4 (0.021)
96-168	8	-0.010 (-0.340, 0.330)	0.973	0.0 (> 0.999)
336-504	3	-0.350 (-0.940, 0.240)	0.246	0.0 (0.800)
Type of graft				
Allograft	2	Insufficient data		
Xenograft	16	-0.230 (-0.480, 0.010)	0.065	0.0 (0.253)
Number of transplanted cells				
< 5 × 10 ⁵	9	-0.430 (-0.940, 0.080)	0.098	45.5 (0.027)
≥ 5 × 10 ⁵	9	-0.200 (-0.510, 0.120)	0.219	0.0 (0.929)
Administration of immunosuppressive				
Not administered	7	-0.620 (-1.390, 0.150)	0.115	32.3 (0.014)
Cyclosporine	11	-0.170 (-0.450, 0.100)	0.221	0.0 (0.970)
Follow-up duration (week)				
≤ 4	9	-0.610 (-1.030, -0.190)	0.005	25.3 (0.060)
> 4	9	-0.010 (-0.330, 0.310)	0.949	0.0 (> 0.999)

CI: Confidence interval; MCAO: Middle cerebral artery occlusion; CCAO: Common carotid artery occlusion; SMD: Standardized mean difference

Neuroprotection: Pooled data analysis indicated that the area demonstrating NeuN⁺ staining in the cortex was not significantly different between ESC-NSC-treated group compared to the untreated stroke group (SMD = 7.45; 95% CI: -2.03 to 16.93; P = 0.124; I² = 98.69%) (Figure 5). Subgroup analysis was not applicable due to limited data in this part of the study.

Inflammation in response to the grafted cells: Meta-analysis from 3 studies showed a lack of difference in the microglia/macrophage marker Iba-1 amongst treatment and control groups (SMD = -3.69; 95% CI: -10.03 to 2.64; P = 0.254; I² = 97.30%). This finding indicates that there is no change in reactive inflammation in response to the grafted cells (Figure 5). Subgroup analysis was not applicable due to limited data in this part of the study.

Publication bias: Modified Egger's test showed no evidence of publication bias in the efficacy of ESC-NSC on locomotion recovery (P = 0.635), neurological deficit (P = 0.896), cylinder test

(P = 0.961), adhesive removal test (P = 0.520), infarct size (P = 0.150), astrogliosis (P = 0.361), neuroprotection (P = 0.313), and inflammation (P = 0.169) (Supplementary).

Certainty of evidence: The certainty of evidence regarding the protective effects of ESC-NSC in ischemic stroke was determined as moderate in locomotion recovery, cylinder test, infarct size, and astrogliosis. The level of evidence for NSS/mNSS, adhesive removal test, neuroprotection, and inflammation was determined as low due to being rated down for presence of inconsistency and risk of bias (Table 5).

Discussion

The aim of the present meta-analysis was to gather and review the available preclinical evidence regarding the efficacy of ESC-NSC administration in functional recovery, neurological status, infarct size, astrogliosis, neuroprotection, and inflammation following ischemic stroke.

Table 5. The certainty of evidence regarding the effect of embryonic stem cell-derived neural stem/progenitor cells (ESC-NSCs) transplantation on ischemic stroke

Outcome	Number of experiments	Risk of bias	Imprecision	Inconsistency (I ² range)	Indirectness	Publication bias	Level of evidence
Locomotion recovery	14	Serious	Not serious	Not serious	Not serious	No	Moderate
NSS/mNSS	5	Serious	Not serious	Serious	Not serious	No	Low
Cylinder test	7	Serious	Not serious	Not serious	Not serious	No	Moderate
Adhesive removal test	4	Serious	Not serious	Serious	Not serious	No	Low
Infarct size	18	Serious	Not serious	Not serious	Not serious	No	Moderate
Astrocytosis	4	Serious	Not serious	Not serious	Not serious	No	Moderate
Neuroprotection	4	Serious	Not serious	Serious	Not serious	No	Low
Inflammation	3	Serious	Not serious	Serious	Not serious	No	Low

NSS: Neurological severity scale; mNSS: Modified neurological severity score

Preclinical evidence showed that the administration of ESC-NSC improved functional recovery and neurological status of animals with ischemic stroke. In addition, transplantation of these cells shortly after ischemia seems to reduce the infarct size and subside the astrocytosis, with no significant effect on neuroprotection and inflammation. The improvement in locomotion recovery was evident in both adult and neonate animals with transient or permanent ischemia, but only if ESC-NSC was administered 24 to 72 hours after injury.

Since the middle cerebral artery (MCA) supplies blood flow to a larger portion of the brain tissue compared to other cerebral arteries, its occlusion is associated with extensive ischemic damage.³⁸ The main areas of the brain that are supplied through MCA are the corticospinal tract and the corticoreticulospinal tract, which are involved in fine manual dexterity and maintaining postural stability.³⁹⁻⁴¹ Therefore, ischemic damage due to occlusion of MCA is expected to affect locomotion more than other brain functions. It should be noted that the occurrence of ischemia in brain tissue (stroke) is associated with the loss of two million brain cells per minute.⁴² Accordingly, the continuation of ischemia every minute will have unpredictable consequences. A significant finding of this study is that ESC-NSC administration in animals subjected to a permanent model has also a significant effect on improving locomotion. Stem cell rejection following allogeneic transplantation into host tissue has always been one of the challenges of reconstructive medicine. Mesenchymal stem cells (MSCs) have low immunogenicity, because they often lack major histocompatibility complex (MHC) class II and costimulatory molecule

expression. On the other hand, they inhibit T cells both directly – by inhibiting natural killer (NK), CD4⁺, and CD8⁺ T cells – and indirectly through dendritic cell modulation. Finally, they create a suppressive microenvironment rich in prostaglandins and interleukin-10 (IL-10); therefore, their allogeneic grafting is not associated with rejection.⁴³⁻⁴⁵ However, this is not the case for other stem cells, including NSCs, and the rejection of these cells due to the role of human leukocyte antigens (HLA) in allogeneic transplantation has always been considered.^{46,47} Surprisingly, our findings suggest that in studies using cyclosporine as the immunosuppressive agent, grafting of ESC-NSCs did not significantly improve locomotion recovery. This observation may initially appear counterintuitive, given the anticipated regenerative capabilities of ESC-NSCs. However, it is important to consider that this could be an incidental finding, primarily due to the limited number of studies available for analysis. The inconclusive nature of this result is further evidenced by the proximity of the lower limit of the CI to zero, indicating a near-neutral effect size. This suggests that while the data do not robustly confirm the efficacy of ESC-NSC transplantation in the presence of cyclosporine, they also do not definitively rule it out. Further research with a larger dataset is crucial to clarify the impact of cyclosporine on the therapeutic benefits of ESC-NSC transplantation in ischemic stroke recovery.

Although neonatal stroke is relatively uncommon, it represents an important cause of lifelong neurological disability, including cerebral palsy (CP) and developmental delay. Several preclinical studies have employed neonatal hypoxic-ischemic models to explore the therapeutic potential of ESC-NSC in immature

brains. Daadi et al. and Shinoyama et al. reported that transplantation in neonatal rodents enhanced axonal sprouting, modulated microglial activation, and improved motor recovery, indicating that the developing brain provides a receptive and plastic environment for cellular repair.^{22,36} More recently, Ji et al. demonstrated that human ESC-derived striatal neurons could reconstruct host neural circuits and restore motor function in neonatal hypoxic-ischemic injury, providing mechanistic evidence of functional integration.⁷ Inclusion of neonatal studies in our meta-analysis, therefore, reflects a biologically distinct yet clinically relevant setting that may amplify the restorative effects of ESC-NSCs. Nevertheless, subgroup analysis confirmed that treatment efficacy persisted in adult animals, suggesting that inclusion of neonatal models did not confound the overall estimates of therapeutic benefit.

The interval between stroke induction and ESC-NSC transplantation influenced treatment efficacy on locomotor recovery and infarct reduction. Although the optimal timing remains debated, our subgroup analysis identified 24-72 hours post-stroke as yielding the largest pooled effects for both outcomes. This aligns with Daadi et al., who transplanted at 24 hours and observed improved motor function, axonal sprouting, and upregulation of trophic and repair genes [glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor 1 (IGF1), fibroblast growth factor 2 (FGF2), C-X-C chemokine receptor type 4 (CXCR4), myelin basic protein (MBP)] alongside microglial modulation – supporting a paracrine mechanism in this window.²² At ~48 hours, Guan et al. reported cell survival without behavioral improvement, likely due to tumorigenesis exacerbated by cyclosporine and ischemic conditions, highlighting the importance of cell purification and immunosuppression control.³¹ In contrast, 7-day transplants also enhanced outcomes and, via sex-determining region Y-related high-mobility-group box 1 (SOX1)⁺ selection, reduced tumorigenic risk, consistent with processes occurring during later remodeling, rather than contradicting the stronger meta-analytic signal at 24-72 hours.^{24,25} Immediate post-stroke transplantation presents additional challenges: reperfusion injury and blood-brain barrier (BBB) disruption can cause extensive graft loss, while stem cell preparation itself requires time. Thus, a 24-hour delay may be both biologically protective and clinically practical.

During this acute-subacute phase, reperfusion injury, BBB breakdown, and immune infiltration drive neuronal damage.⁴⁸⁻⁵⁰ Interventions such as NSC therapy aim to counteract these processes.^{6, 51-53} Through anti-inflammatory, pro-angiogenic,^{54, 55} and neurogenic⁵⁶⁻⁵⁸ effects, NSCs promote functional recovery, which is consistent with our findings of improved outcomes following early-phase administration.

All studies in the present review employed intracerebral transplantation, precluding comparison across alternative delivery routes. This method enables precise delivery to the ischemic region, facilitating stem cell migration, proliferation, and differentiation within the lesion.¹¹ Chang et al. demonstrated extensive migration and neurological improvement following intracerebral transplantation of ESC-NSCs, supporting the feasibility and effectiveness of localized delivery for targeted engraftment.²⁰ However, this approach is inherently invasive and technically demanding, requiring stereotaxic surgery and immunosuppression, with potential risks of graft overgrowth or immune reaction. Thus, despite its experimental efficacy, the intracerebral route poses significant translational and scalability limitations compared with less invasive systemic approaches.

We rated down the level of evidence for all investigated outcomes, due to possible risk of bias. However, recent discussions suggest that current guidelines for evaluating the risk of bias in preclinical research may not align well with best practices for conducting such studies.⁵⁹ Consequently, the risk of bias might be overestimated in certain domains simply due to insufficient reporting, as some details may not have been documented by the authors in their manuscripts.

In the present meta-analysis, while some funnel plots appear asymmetric, the modified Egger's test did not indicate significant publication bias. It is important to note that asymmetry in funnel plots can result from factors other than publication bias. Here we present some potential explanations for the observed asymmetry to be considered. Funnel plot asymmetry may be driven by methodological heterogeneity among the included studies. Variability in animals' age and cerebral ischemia models, intervention protocols, or outcome definitions can contribute to differences in effect sizes, causing asymmetry in the funnel plot.⁶⁰ For example, our findings indicate that studies employing transient MCAO (tMCAO) with

different durations of occlusion (7 to 180 minutes) reported varying effect sizes for locomotion recovery, as shown in the subgroup analysis. Differences in the timing of ESC-NSC administration post-stroke, ranging from 24 to 72 hours, also influenced the pooled effect sizes, contributing to the heterogeneity.

Asymmetric funnel plots may also arise due to variations in study quality. In animal studies, differences in quality could stem from inconsistencies in randomization, blinding, or adherence to standard protocols. For instance, only 9 out of the 23 studies clearly reported allocation concealment, and caregiver blinding was not disclosed in any of the included studies. These methodological inconsistencies could have contributed to variability in effect estimates and potential asymmetry in the funnel plots.

An important limitation to note regarding ESC-NSC transplantation is the potential for tumorigenesis. Some studies have reported tumor formation following intracerebral injection of ESC-NSCs and have proposed various strategies – such as modifying culture conditions or adding adjunctive agents – to mitigate this risk, which remains a major challenge for clinical translation.^{21,61} Moreover, while neuroinflammation in this review was primarily assessed using Iba-1 as a microglial activation marker, this approach may not fully capture the complexity of inflammatory responses. Iba-1 reflects shifts between pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes; however, other markers and cytokine profiles would be needed for a more comprehensive evaluation.

Another limitation is the presence of small-sample studies and potential outliers, which can influence pooled estimates. To address this, forest and Galbraith plots were inspected to identify influential studies, and leave-one-out sensitivity analyses were performed. The pooled results

remained consistent, suggesting robustness despite small-sample variability. Additionally, although several pooled outcomes exhibited substantial heterogeneity, subgroup analyses and random-effects modeling were applied to explore and account for between-study variability. Nevertheless, the considerable methodological diversity and limited sample sizes across studies warrant cautious interpretation of these findings.

Future studies should incorporate broader inflammatory markers and address both tumorigenic potential and immunological dynamics to enhance the translational relevance of ESC-NSC therapy in stroke.

Conclusion

Based on low-to-moderate certainty evidence from preclinical models, ESC-NSC transplantation is associated with improvements in locomotor/neurological outcomes and a reduction in infarct size. Signals of greater benefit were observed when cells were administered 24-72 hours after stroke. However, given small sample sizes, risk of bias, and between-study heterogeneity, these findings should be interpreted with caution and regarded as hypothesis-generating. Further rigorously designed, blinded, and adequately powered studies – including translational and early-phase clinical research – are needed to confirm efficacy and define optimal timing.

Conflict of Interests

The authors declare no conflict of interest in this study.

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