Rovedar

JLAR

Journal of Lab Animal Research. 2026; 5(4): 75-81.

DOI: 10.58803/jlar.v5i4.119

http://jlar.rovedar.com/

Original Article

Effects of Human Umbilical Cord Mesenchymal Stem Cell-Derived Exosomes on an Ischemic Stroke Rat Model

Mansooreh Miri Moghaddam[1], Ali Heydari[2], Kian Ale Mohammad[3], Parichehr Ebrahimi Shahabadi[4], Mahdi Falsafi[5], Seyed Ali Shariat Razavi[6], Parsa Jahani[7], Melika Abedini[8], and Sima Saravani[9*]

[1] Doctor of Veterinary Medicine, Faculty of Clinical Sciences, Shahid Bahonar University of Kerman, Kerman, Iran
[2] Department of Clinical Science, Veterinary Faculty, Karaj Branch, Islamic Azad University, Karaj, Iran
[3] Department of Veterinary Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran
[4] Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
[5] Doctor of Veterinary Medicine, Small Animal Clinician, Tehran, Iran
[6] Department of Neuroscience, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[7] Department of Clinical Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
[8] Doctor of Veterinary Medicine graduate, Shahid Chamran University of Ahvaz, Ahvaz, Khuzestan Province, Iran
[9] Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol, Iran

*** Corresponding author:** Sima Saravani, Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol, Iran. Email: simasarvani@yahoo.com

ARTICLE INFO

Article History:
Received: 12/05/2026
Revised: 17/06/2026
Accepted: 20/07/2026
Published: 30/08/2026

check for updates

Keywords:
Cerebral artery occlusion
Immunomodulation
Inflammation
Neuroprotection

ABSTRACT

Introduction: Ischemic stroke remains a leading cause of disability worldwide, with limited therapeutic options beyond the acute stage. Mesenchymal stem cell (MSC)-derived exosomes have emerged as a promising cell-free therapeutic strategy due to their immunomodulatory and neurotrophic properties. The current study aimed to evaluate the neuroprotective effects of human umbilical cord MSC-derived exosomes (hUC-MSC-Exos) on neurological function, infarct volume, and inflammatory response in a rat model of middle cerebral artery occlusion (MCAO).

Materials and methods: A total of 36 adult male rats weighing 250-300 grams and aged 8-10 weeks were randomly divided into three groups of 12 rats each. The first group underwent surgery without occlusion (Group A). The second group consisted of MCAO rats that received 100 µL of phosphate-buffered saline (PBS) intravenously (Group B). The third group consisted of MCAO rats that received 100 µg of hUC-MSC-Exos (1×10^{10} particles), diluted in 100 µL of sterile PBS, administered intravenously (Group C). Ischemic stroke was induced by 60-minute transient MCAO followed by reperfusion. Neurological deficits were assessed using the modified neurological severity score (mNSS) and grip strength test at 24 hours, 48 hours, and 7 days post-stroke. On day 7, brains were harvested for 2,3,5-triphenyltetrazolium chloride (TTC) staining to measure infarct volume, and cortical tissue was collected to measure tumor necrosis factor-alpha (TNF-α) and Interleukin-10 (IL-10) levels by enzyme-linked immunosorbent assay (ELISA).

Results: Administration of 100 µg of hUC-MSC-Exos significantly improved neurological function compared To Group B, as evidenced by reduced mNSS scores from 12.4 ± 1.2 to 11.8 ± 1.4 in 24 hours, from 11.2 ± 1.3 to 8.3 ± 1.1 in 48 hours, and from 9.6 ± 1.5 to 4.5 ± 0.9 on day 7, along with increased grip strength. Moreover, TTC staining revealed a significant reduction in infarct volume in Group C compared to the control group. The pro-inflammatory cytokine TNF-α and IL-10 in the ischemic cortical tissue in Group C were significantly decreased compared to Group B.

Conclusion: The systemic administration of hUC-MSC-Exos had neuroprotective effects in rats by reducing infarct size, improving motor function, and modulating the inflammatory response.

1. Introduction

Ischemic stroke is one of the primary causes of death and long-term disability worldwide[1]. Approximately 80-85% of all strokes are ischemic, resulting from the occlusion of blood vessels supplying the brain, resulting in a severe reduction in

Cite this paper as: Miri Moghaddam M, Heydari A, Ale Mohammad K, Ebrahimi Shahabadi P, Falsafi M, Shariat Razavi SA, Jahani P, Abedini M, and Saravani S. Effects of Human Umbilical Cord Mesenchymal Stem Cell-Derived Exosomes on an Ischemic Stroke Rat Model. 2026; 5(4): 75-81. DOI: 10.58803/jlar.v5i4.119

oxygen and nutrients to the neural tissue[2]. An ischemic stroke initiates a complex cascade of cellular reactions, including oxidative stress, extensive inflammation, and ultimately apoptosis or necrosis of neural cells, occurring not only in the lesion area but also in more distant regions[3,4]. Given the aging population and increasing cardiovascular risk factors, the economic and social burden of stroke is increasing, highlighting the need for more effective treatment[5].

Currently, the only approved treatment for the acute phase of ischemic stroke is the administration of thrombolytic medicines (alteplase or tPA) within the golden window of 4 to 4.5 hours or mechanical removal of the clot using thrombectomy[6]. Furthermore, the return of blood to ischemic tissue (reperfusion) can itself damage neurons by generating reactive oxygen species and amplifying inflammation[7]. Therefore, neuroprotective therapies that can interrupt the chain of cell death at an early stage and salvage penumbra tissue have been proposed as a vital complementary approach in stroke management[8].

Over the past decade, mesenchymal stem cells derived from different tissues, particularly umbilical cord tissue, have garnered significant attention owing to their accessibility, reduced immunogenicity, and limited differentiation potential[9,10]. Umbilical cord mesenchymal stem cells are mediated not primarily by their ability to replace damaged cells, but rather by the secretion of paracrine factors, including growth factors, anti-inflammatory molecules, and, especially, extracellular vesicles (exosomes)[11]. Exosomes are nanoparticles ranging from 30 to 150 nm that transport diverse biological molecules, including microRNAs, proteins, and lipids, and are crucial in intercellular communication and regulating inflammation responses[12]. The utilization of exosomes derived from stem cells as a cell-free therapeutic approach presents considerable benefits compared to the direct injection of living cells[13-15], These benefits include the lack of tumor formation risk, the absence of severe immune reactions resulting from the transplantation of heterogeneous cells, increased ease of storage and transportation, and the capability to cross the blood-brain barrier owing to their nanometer size[16]. Previous studies in stroke animal models indicated that injecting exosomes derived from umbilical cord stem cells can improve neurological function by reducing apoptosis, stimulating angiogenesis, and inhibiting the migration of inflammatory cells into damaged tissue[17-19].

On the other hand, simple clinical assessments, such as behavioral analyses, lesion volume quantification by standard histological methods, and principal inflammatory indicators, including tumor necrosis factor-alpha (TNF-α) and Interleukin-10 (IL-10), measured via enzyme-linked immunosorbent assay (ELISA) kits, can yield essential insights regarding the overall efficacy of exosomes[20]. Accordingly, the present study aimed to determine the neuroprotective effects of intravenous administration of human umbilical cord mesenchymal stem cell-derived exosomes (hUC-MSC-Exos) on the severity of neurological deficits, grip strength, final cerebral infarct volume, and changes in TNF-α and IL-10 levels in the cerebral cortex tissue of ischemic stroke rats.

2. Materials and Methods

2.1. Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed, and the authors confirmed that the study was carried out in compliance with the ARRIVE guidelines.

2.2. Animals and grouping

In the present study, 36 adult male Wistar rats, aged 8-10 weeks and weighing 250-300 g, were obtained from Razi Animal Laboratory in Tehran, Iran. All rats were housed under established laboratory conditions, with a 12-hour light/dark cycle, temperature maintained at 22 ± 2°C, and humidity between 50% and 60%. Rats had *ad libitum* access to food and water. After one week of acclimatization, the rats were randomly divided into three experimental groups, with 12 rats in each group (4 replicates of 3 rats each). The experiment was conducted as a single independent *in vivo* study. The first group was the sham group, in which rats underwent the same surgical procedure without arterial occlusion (Group A), the second group consisted of model of middle cerebral artery occlusion (MCAO) rats, in which ischemic rats received 100 µL of phosphate-buffered saline (PBS) as placebo (Group B), and the third group contained MCAO rats that received 100 µg of hUC-MSC-Exos (approximately 1×10^{10} particles) diluted in 100 µL of sterile PBS (Group C). The PBS and hUC-MSC-Exos were administered intravenously via the tail vein.

2.3. Isolation and characterization of exosomes

The hUC-MSCs were cultured in DMEM/F12 medium (Gibco, Brazil) supplemented with 10% exosome-depleted fetal bovine serum (FBS; Gibco, Brazil) to avoid bovine exosome contamination[21]. After 48 hours, the culture supernatant was collected and subjected to sequential centrifugation to isolate exosomes. Briefly, the supernatant was centrifuged at 300 g for 10 minutes to remove cell debris, then at 2000 g for 20 minutes to eliminate apoptotic bodies, and finally at 10,000 g for 30 minutes to remove large extracellular vesicles. Finally, the supernatant was ultracentrifuged at 100,000 g for 90 minutes at 4°C to pellet the exosomes[22]. The pellet was washed once with PBS and then re-ultracentrifuged under the same conditions. The final exosome pellet was resuspended in 100 µL of sterile PBS. Protein concentration of the isolated exosomes was measured using the bicinchoninic acid (BCA) assay, and the samples were stored at -80°C until further analysis use[23].

2.4. Induction of focal cerebral ischemia

Transient focal cerebral ischemia was induced via the intraluminal filament model of MCAO, following the method described by Zhan et al.[24], with slight modifications. Rats were anesthetized by intraperitoneal injection of 0.20-0.24 mL of a 100 mg/mL ketamine (80 mg/kg; Alfasan, Netherlands) and 0.125-0.15 mL of a 20

mg/mL xylazine (10 mg/kg; Alfasan, Netherlands)[25]. After a midline cervical incision, the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were carefully isolated and dissected from surrounding tissues. A 4-0 monofilament nylon suture (Doccol Corporation, Sharon, MA, USA) with a rounded tip, coated with poly-L-lysine, was inserted through the ECA into the ICA and carefully advanced about 18-20 mm from the carotid bifurcation until slight resistance indicated occlusion of the MCA origin. After 60 minutes of ischemia, the filament was carefully removed to initiate reperfusion. Occlusion success was verified by a notable decrease in regional cerebral blood flow, monitored with laser Doppler flowmetry in some animals, and by observing right-sided Horner's syndrome and forelimb signs flexion. In Group A, the same surgical procedure was performed, but the filament was not advanced into the ICA. Body temperature was maintained at 37°C throughout the surgery using a heating pad.

2.5. Exosome administration and experimental protocol

Immediately following reperfusion (time zero), the rats in Group C were administered a single intravenous injection of hUC-MSC-Exos (comprising 100 µg of exosomal protein diluted in 100 µL of sterile PBS) via the tail vein[26]. A second identical dose was administered 24 hours post-ischemia. Neurological function was assessed at three intervals, 24 hours, 48 hours, and 7 days post-reperfusion, using two behavioral tests, including the modified neurological severity score (mNSS) and the grip strength test[27]. On day 7 post-stroke, all rats were deeply anesthetized with an intraperitoneal overdose of ketamine (300 mg/kg) and xylazine (30 mg/kg)[28], then euthanized by cervical dislocation to confirm death. Brains were quickly removed; the olfactory bulbs and cerebellum were discarded. Each brain was subsequently sectioned into five coronal sections (2 mm thick) for 2,3,5-triphenyltetrazolium chloride (TTC) staining. The ipsilateral cortical tissue surrounding the infarct area was meticulously dissected, snap-frozen in liquid nitrogen, and preserved at -80°C for subsequent ELISA analysis.

2.6. Behavioral assessments, infarct volume measurement, and cytokine assay

2.6.1. Neurological severity score

The mNSS was used to evaluate motor, sensory, reflex, and balance functions on a scale of 0 to 18, where 0 indicated normal neurological severity, and 18 indicated maximal deficit. An investigator blinded to group assignments performed the mNSS analysis. Forelimb grip strength was measured using a simple grip strength meter; each rat was allowed to grasp a horizontal bar and was pulled gently backward until the grip was released for the grip strength test. The maximum force (in grams) was recorded, and the average of three consecutive trials was used for analysis[29,30].

2.6.2. Infarct volume measurement

The 2-mm coronal brain sections were incubated in a 2% TTC solution in PBS at 37°C for 20 minutes in the dark. Viable brain tissue was stained deep red, whereas ischemic (infarcted) tissue remained unstained (pale white)[31]. Following staining, the sections were fixed in 4% paraformaldehyde (Ercros, Spain) for 24 hours and subsequently photographed with a digital camera. Infarct areas were quantified using ImageJ software (NIH, USA) for each coronal section. The total infarct volume was calculated by adding the infarct areas and multiplying by the section thickness (2 mm). It was then expressed as a percentage of the contralateral hemisphere volume to account for cerebral edema.

2.6.3. Cytokine measurement

Frozen cortical tissue samples from the ischemic penumbra region were homogenized in radio-immunoprecipitation assay (RIPA) lysis buffer containing protease inhibitors. Following centrifugation at 12,000×g for 20 minutes at 4°C, the supernatant was collected, and the total protein concentration was subsequently determined using a BCA assay. The levels of TNF-α and IL-10 were measured in tissue homogenates using a commercial rat-specific ELISA kit (Arigo Biolaboratories, Taiwan), following the manufacturer's instructions[32]. All samples were processed in duplicate, with results expressed as picograms of cytokine per milligram of total protein (pg/mg protein).

2.7. Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software (version 21). Comparisons among the three groups were conducted using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. For behavioral tests with repeated measurements over time (mNSS and grip strength), two-way repeated-measures ANOVA was used, followed by Bonferroni's post hoc test. A p-value of less than 0.05 was considered statistically significant. Investigators performed all experiments and analyses while blinded to group allocation.

3. Results

3.1. Neurological function assessment

At 24 hours after ischemia, no significant difference was observed between Group B (12.4 ± 1.2) and Group C (11.8 ± 1.4) in mNSS scores ($p > 0.05$). However, at 48 hours, Group C had a significantly lower mNSS score (8.3 ± 1.1) than Group B (11.2 ± 1.3; $p < 0.05$). This improvement persisted through day 7, with Group C scoring 4.5 ± 0.9 compared with 9.6 ± 1.5 in Group B ($p < 0.05$).

Similarly, grip strength in Group C (185.4 ± 22.3 g) was significantly higher than in Group B (132.6 ± 18.7 g) at 48 hours ($p < 0.05$) and on day 7 (245.7 ± 19.5 g compared to

148.3 ± 21.4 g, respectively; $p < 0.05$). At 24 hours, grip strength did not differ significantly between Group B (108.4 ± 16.3 g) and Group C (115.6 ± 19.7 g; $p > 0.05$).

Group A maintained normal neurological function throughout the study, with an mNSS score of 0.5 ± 0.2 and grip strength of 310.2 ± 15.8 g at day 7, confirming the validity of the MCAO model (Table 1).

Table 1. Neurological function assessment across exosome-treated and untreated ischemic rats aged 8-10 weeks old

Groups	Time point	mNSS score	Grip strength (g)	P-value
Group A	24 hours / 48 hours / 7 days	0.5 ± 0.2	310.2 ± 15.8	-
Group B	24 hours	12.4 ± 1.2[a]	108.4 ± 16.3[a]	-
Group B	48 hours	11.2 ± 1.3[a]	132.6 ± 18.7[a]	-
Group B	7 days	9.6 ± 1.5[a]	148.3 ± 21.4[a]	-
Group C	24 hours	11.8 ± 1.4[a]	115.6 ± 19.7[a]	> 0.05
Group C	48 hours	8.3 ± 1.1[b]	185.4 ± 22.3[b]	< 0.05
Group C	7 days	4.5 ± 0.9[b]	245.7 ± 19.5[b]	< 0.05

mNSS: Modified neurological severity score. Group A (Sham group): Rats underwent the same surgical procedure without arterial occlusion, Group B: MCAO rats received 100 µL of PBS, Group C: MCAO rats received 100 µg of hUC-MSC-Exos diluted in 100 µL of sterile PBS. [a,b] Mean values with different superscript letters within the same column and time point indicate significant differences ($p < 0.05$). P-values in the final column represent comparisons between Group C and Group B at each corresponding time point. - indicates not applicable (no p-value calculated for sham group).

3.2. Infarct volume measurement

The TTC staining performed on day 7 post-stroke revealed distinct pale-white infarct areas in the ipsilateral hemisphere of ischemic rats, whereas the contralateral hemisphere and the brains of sham-operated animals (Group A) were uniformly stained deep red. The mean infarct volume in Group B was 28.6 ± 4.3% of the contralateral hemisphere, indicating a substantial ischemic lesion. In contrast, Group C exhibited a significantly reduced infarct volume of 14.2 ± 3.1% compared to Group B ($p < 0.05$), representing approximately a 50% reduction in lesion size. No infarct was observed in Group A (Table 2).

Table 2. Infarct volume results across exosome-treated and untreated ischemic rats at day 7 of the experiment

Group	Infarct volume (percentage of contralateral hemisphere)	P-value
Group A	0	-
Group B	28.6 ± 4.3[a]	-
Group C	14.2 ± 3.1[b]	p < 0.05

Group A (Sham group): Rats underwent the same surgical procedure without arterial occlusion, Group B: MCAO rats received 100 µL of PBS, Group C: MCAO rats received 100 µg of hUC-MSC-Exos diluted in 100 µL of sterile PBS. [a,b] Mean values with different superscript letters within the same column indicate significant differences ($p < 0.05$). - indicates not applicable (no p-value calculated for sham group).

3.3. Inflammatory cytokine levels in brain tissue

The ELISA analysis of cortical tissue homogenates from the ischemic penumbra region revealed a significant imbalance in inflammatory mediators between the groups. Group B had significantly higher TNF-α levels (78.4 ± 12.6 pg/mg protein) compared to Group A (18.3 ± 4.2 pg/mg protein; $p < 0.05$), indicating a strong neuroinflammatory response to ischemic injury. Treatment with hUC-MSC-Exos significantly reduced the inflammatory response, as evidenced by a reduction in TNF-α levels to 42.7 ± 9.8 pg/mg protein in Group C compared with Group B ($p < 0.05$).

Conversely, IL-10 exhibited an opposite pattern relative to TNF-α. Group C had significantly higher IL-10 levels (65.3 ± 11.4 pg/mg protein) than Group B (31.8 ± 7.6 pg/mg protein) and Group A (25.1 ± 5.3 pg/mg protein; $p < 0.05$). No significant difference was observed between Group A and Group B for IL-10 level ($p > 0.05$; Table 3).

Table 3. Cytokine expression analysis in brain cortical homogenates across exosome-treated and untreated ischemic rats at day 7 of the experiment

Group	TNF-α (pg/mg protein)	IL-10 (pg/mg protein)	P-value
Group A	18.3 ± 4.2[c]	25.1 ± 5.3[b]	-
Group B	78.4 ± 12.6[a]	31.8 ± 7.6[b]	-
Group C	42.7 ± 9.8[b]	65.3 ± 11.4[a]	p < 0.05

Group A (Sham group): Rats underwent the same surgical procedure without arterial occlusion, Group B: MCAO rats received 100 µL of PBS, Group C: MCAO rats received 100 µg of hUC-MSC-Exos diluted in 100 µL of sterile PBS. [a,b,c] Mean values with different superscript letters within the same column indicate significant differences ($p < 0.05$). - indicates not applicable (no p-value calculated for sham group).

3.4. Correlation analysis between inflammatory markers and functional outcomes

A strong positive correlation was observed between TNF-α levels and infarct volume ($r = 0.82$, $p < 0.05$), and between TNF-α levels and mNSS scores ($r = 0.79$, $p < 0.05$), indicating that higher pro-inflammatory activity was associated with more severe brain damage and poorer neurological outcomes. Additionally, IL-10 levels demonstrated a significant negative correlation with infarct volume ($r = -0.71$, $p < 0.05$) and mNSS scores ($r = -0.68$, $p < 0.05$), suggesting that higher anti-inflammatory cytokine levels were associated with smaller lesions and improved neurological function (Table 4).

Table 4. Correlation between inflammatory cytokines and neurological outcomes

Correlation pair	Correlation coefficient (r)	P-value	Type of correlation
TNF-α versus infarct volume	+0.82	$p < 0.05$	Strong positive
TNF-α versus mNSS Score	+0.79	$p < 0.05$	Strong positive
IL-10 versus infarct volume	−0.71	$p < 0.05$	Significantly negative
IL-10 versus mNSS Score	−0.68	$p < 0.05$	Significantly negative

TNF-α: Tumor necrosis factor-alpha, IL-10: Interleukin-10, mNSS: Modified neurological severity score, MCAO: Middle cerebral artery occlusion, PBS: phosphate-buffered saline

4. Discussion

The current findings indicated that administering 100 µg of exosomal protein in 100 µL of PBS as 2 doses (immediately and 24 hours after ischemia) via the tail vein in a rat ischemic stroke model notably reduced cerebral infarct volume and improved neurological function. The hUC-MSC-Exos modulated the inflammatory response by reducing TNF-α and increasing IL-10 in brain tissue. Consistent with the present results, umbilical cord stem cell-derived exosomes, as a cell-free therapeutic strategy, had remarkable neuroprotective effects in ischemic stroke by modulating immune responses[33,34].

The present results demonstrated that injection of hUC-MSC exosomes notably reduced neurological deficit scores and increased grip strength at 24 hours, 48 hours, and 7 days after reperfusion. Functional improvement was accompanied by a 50% reduction in infarct volume, as determined by TTC staining on day 7. The current findings indicated that exosomes not only prevented neuronal cell death in the lesion area but also preserved and restored penumbral function. Shen et al.[35] demonstrated that early administration of umbilical cord stem cell-derived exosomes in an MCAO mouse model notably improved neuromuscular function scores and reduced infarct volume, which was consistent with the present results. Moreover, Consistent with the present results, Barbati et al.[36] reported that intranasal administration of extracellular vesicles 48 hours post-stroke and twice weekly for four weeks, derived from bone marrow mesenchymal stem cells, remarkably improved forelimb motor deficits in a mouse model of cortical stroke, accompanied by reduced infarct volume and astrogliosis.

The ELISA results in the present study demonstrated that exosomes notably reduced TNF-α and increased IL-10 in cortical tissue. The observed changes indicated a transition in cytokine balance from a pro-inflammatory to an anti-inflammatory state. Consistent with current findings, Shan et al.[37] explicitly demonstrated that exosomes derived from mesenchymal stem cells modulated inflammatory responses in stroke by decreasing pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, and elevating anti-inflammatory cytokines such as IL-10 and TGF-β. Furthermore, Liu et al.[38] reported that exosomes derived from umbilical cord stem cells mitigated hypoxic cerebrovascular endothelial injury by inhibiting the AMPK/NLRP3 pathway and suppressing GSDMD-mediated pyroptosis, potentially accounting for the reduced inflammation and enhanced functional outcomes observed in the present study. The strong correlation between TNF-α reduction and infarct volume reduction, as well as the negative correlation between IL-10 and mNSS scores, confirmed the key role of immunomodulation in the protective effects of exosomes.

Shen et al.[35] administered a single intravenous dose of 100 µg of hUC-MSC-derived exosomes 6 hours after MCAO in an ischemic stroke mouse model, notably reducing inflammatory factors such as Ccl2, Ccl5, Il-1β, Il-6, and TNF-α. Both studies, despite using different cell sources, highlighted a shared immunomodulatory mechanism as the main basis for their therapeutic effects. Furthermore, a systematic review by Chen et al.[39] on the regulatory role of stem cell exosomes in the inflammatory response to stroke demonstrated that exosomes exerted protective effects by promoting microglial polarization from an M1 (inflammatory) to an M2 (reparative) phenotype and by increasing IL-10 secretion, findings consistent with the present results.

5. Conclusion

The present study demonstrated that intravenous injection of human umbilical cord mesenchymal stem cell-derived exosomes in a rat ischemic stroke model was associated with a significant reduction in cerebral infarct volume, improved neurological function, and modulation of the inflammatory response, as evidenced by decreased TNF-α and increased IL-10. The current findings indicated that the exosome-based cell-free therapeutic approach is a safe, accessible, and effective strategy for neuroprotection in stroke. Given the high prevalence of stroke and the limitations of existing treatments, umbilical cord stem cell-derived exosomes could be considered as a potential complementary or alternative option in the near future. However, the generalizability of the current results was limited by the use of a single animal model, short follow-up period, and lack of in-depth molecular analysis. Future studies should explore dose optimization, long-term efficacy, molecular mechanisms, alternative routes of administration, safety profiles, and combination therapies to facilitate clinical translation.

Declarations

Authors' contributions

Mansooreh Miri Moghaddam contributed to the study design, performed the experimental procedures, and drafted the manuscript, Ali Heydari was involved in the preparation and characterization of the therapeutic agent, Kian Ale Mohammad and Parichehr Ebrahimi Shahabadi conducted the laboratory analyses and data collection, Mahdi Falsafi performed the statistical analysis and prepared the figures and tables, Seyed Ali Shariat Razavi and Parsa Jahani assisted in animal handling, sample collection, and literature review,

Melika Abedini supported the experimental procedures and laboratory logistics, and Sima Saravani supervised the entire study, provided scientific guidance, and finalized the manuscript. All authors reviewed and approved the final edition of the manuscript.

Availability of data and materials

All datasets are available upon request from the corresponding author.

Competing interests

The authors declared that they have no competing interests.

Ethical considerations

All the authors confirmed that ethical concerns, such as plagiarism, permission to publish, research misconduct, data fabrication or falsification, duplicate submissions, and redundant publication, have been thoroughly reviewed. The authors declared that artificial intelligence (AI) tools (ChatGPT Version 4) were used solely for language polishing and grammatical correction during the preparation of this manuscript, and after that, the authors reviewed and edited the language-polished version of the manuscript. No AI tools were employed for data generation, analysis, interpretation, or scientific decision-making. The authors have critically reviewed and verified all content and take full responsibility for the accuracy, originality, and integrity of the final manuscript, including all data, figures, and conclusions. This responsibility applies to the initial submission, all revisions, and the final publication.

Funding

No external funding was received for the present study.

References

1. Pu L, Wang L, Zhang R, Zhao T, Jiang Y, Han L, et al. Projected global trends in ischemic stroke incidence, deaths and disability-adjusted life years from 2020 to 2030. Stroke. 2023; 54(5): 1330-1339. DOI: 10.1161/STROKEAHA.122.040073
2. Ahmed Z, Chaudhary F, and Agrawal DK. Epidemiology, pathophysiology, and current treatment strategies in stroke. Cardiol Cardiovasc Med. 2024; 8(4): 389-404. DOI: 10.26502/fccm.92920399
3. Shi K, Tian DC, Li ZG, Ducruet AF, Lawton MT, Shi FD, et al. Global brain inflammation in stroke. Lancet Neurol. 2019; 18(11): 1058-1066. DOI: 10.1016/S1474-4422(19)30078-X
4. Sekerdag E, Solaroglu I, and Gursoy-Ozdemir Y. Cell death mechanisms in stroke and novel molecular and cellular treatment options. Curr Neuropharmacol. 2018; 16(9): 1396-1415. DOI: 10.2174/1570159X16666180302115544
5. Ovbiagele B, Goldstein LB, Higashida RT, Howard VJ, Johnston SC, Khavjou OA, et al. Forecasting the future of stroke in the United States: A policy statement from the American Heart Association and American Stroke Association. Stroke. 2013; 44(8): 2361-2375. DOI: 10.1161/STR.0b013e31829734f2
6. Herpich F, and Rincon F. Management of acute ischemic stroke. Crit Care Med. 2020; 48(11): 1654-1663. DOI: 10.1097/CCM.0000000000004597
7. Wu L, Xiong X, Wu X, Ye Y, Jian Z, Zhi Z, et al. Targeting oxidative stress and inflammation to prevent ischemia-reperfusion injury. Front Mol Neurosci. 2020; 13: 28. DOI: 10.3389/fnmol.2020.00028
8. Fisher M, and Savitz SI. Pharmacological brain cytoprotection in acute ischaemic stroke renewed hope in the reperfusion era. Nat Rev Neurol. 2022; 18(4): 193-202. DOI: 10.1038/s41582-021-00605-6
9. Fan CG, Zhang Qj, and Zhou Jr. Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. Stem Cell Rev Rep. 2011; 7(1): 195-207. DOI: 10.1007/s12015-010-9168-8
10. El Omar R, Beroud J, Stoltz J-F, Menu P, Velot E, Decot V, et al. Umbilical cord mesenchymal stem cells: The new gold standard for mesenchymal stem cell-based therapies?. Tissue Eng PartB Rev. 2014; 20(5): 523-544. DOI: 10.1089/ten.teb.2013.0664
11. Nagamura-Inoue T, and He H. Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility. World J Stem Cells. 2014; 6(2): 195-202. DOI: 10.4252/wjsc.v6.i2.195
12. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, and Kim JH. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. Cells. 2019; 8(4): 307. DOI: 10.3390/cells8040307
13. Zhang L, Yang F, Liu J, Li M, Mao F, Ocansey D, et al. Exosomes in tissue engineering and cell-free therapy: A comprehensive review. Eur Cell Mater. 2026; 55: 80-105. DOI: 10.22203/eCM.v055a06
14. Ma ZJ, Yang JJ, Lu YB, Liu ZY, and Wang XX. Mesenchymal stem cell-derived exosomes: Toward cell-free therapeutic strategies in regenerative medicine. World J Stem Cells. 2020; 12(8): 814-840. DOI: 10.4252/wjsc.v12.i8.814
15. Habib SM, Haider KH, and Martini MF. The potential of mesenchymal stem cell-derived exosomes in cell-free therapy for myocardial repair. Handbook of regenerative medicine. 2025. p. 372-389. DOI: 10.1201/9781003611509-24
16. Zakrzewski W, Dobrzyński M, Szymonowicz M, and Rybak Z. Stem cells: Past, present, and future. Stem Cell Res Ther. 2019; 10(1): 68. DOI: 10.1186/s13287-019-1165-5
17. Luan Z, Liu J, Li M, Wang Y, and Wang Y. Exosomes derived from umbilical cord-mesenchymal stem cells inhibit the NF-κB/MAPK signaling pathway and reduce the inflammatory response to promote recovery from spinal cord injury. J Orthop Surg Res. 2024; 19(1): 184. DOI: 10.1186/s13018-024-04651-w
18. Abbaszadeh H, Ghorbani F, Derakhshani M, Movassaghpour A, and Yousefi M. Human umbilical cord mesenchymal stem cell-derived extracellular vesicles: A novel therapeutic paradigm. J Cell Physiol. 2020; 235(2): 706-717. DOI: 10.1002/jcp.29004
19. Zhang ZW, Wei P, Zhang GJ, Yan JX, Zhang S, Liang J, et al. Intravenous infusion of the exosomes derived from human umbilical cord mesenchymal stem cells enhance neurological recovery after traumatic brain injury via suppressing the NF-κB pathway. Open Life Sci. 2022; 17(1): 189-201. DOI: 10.1515/biol-2022-0022
20. Yang Z, Liang Z, Rao J, Xie H, Zhou M, Xu X, et al. Hypoxic-preconditioned mesenchymal stem cell-derived small extracellular vesicles promote the recovery of spinal cord injury by affecting the phenotype of astrocytes through the miR-21/JAK2/STAT3 pathway. CNS Neurosci Ther. 2024; 30(3): e14428. DOI: 10.1111/cns.14428
21. Chang YH, Wu KC, and Ding DC. Chondrogenic potential of human umbilical cord mesenchymal stem cells cultured with exosome-depleted fetal bovine serum in an osteoarthritis mouse model. Biomed. 2022; 10(11): 2773. DOI: 10.3390/biomedicines10112773
22. Wang SQ, Jin MJ, Guo ZK, Shen DR, Jin LN, Cheng F, et al. Trace element-dictated exosome modules and self-adaptive dual-network hydrogel orchestrate diabetic foot regeneration through complement-mitochondria-autophagy circuitry. Military Med Res. 2025; 12(1): 71. DOI: 10.1186/s40779-025-00658-4
23. Wang N, Yin M, Yu J, Zhang J, and Pan X. Comparative investigation of exosome extraction from rat bone marrow mesenchymal stem cells using three different methodologies. Electrophoresis. 2024; 45(21-22): 2045-2053. DOI: 10.1002/elps.202400055
24. Zhan C, and Yang J. Protective effects of isoliquiritigenin in transient middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Pharmacol Res. 2006; 53(3): 303-309. DOI: 10.1016/j.phrs.2005.12.008
25. Khalilzadeh E, Azarpey F, Hazrati R, and Saiah GV. Evaluation of different classes of histamine H1 and H2 receptor antagonist effects on neuropathic nociceptive behavior following tibial nerve transection in rats. Eur J Pharmacol. 2018; 834: 221-229. DOI: 10.1016/j.ejphar.2018.07.011
26. Zeng X, Liao Y, Huang D, Yang J, Dai Z, Chen Z, et al. Exosomes derived from hUC-MSCs exhibit ameliorative efficacy upon previous cesarean scar defect via orchestrating β-TrCP/CHK1 axis. Sci Rep. 2025; 15(1):

489. DOI: 10.1038/s41598-024-84689-2
27. Xu L, Feng Y, Zheng X, Wang M, Fang Y, Li L, et al. Astragaloside IV-preconditioned neural stem cell transplantation promotes neurogenesis and functional recovery via the miR-199a-5p/Caveolin-1 pathway in a rat model of ischemic stroke. Int Immunopharmacol. 2026;168:115898. DOI: 10.1016/j.intimp.2025.115898
28. Farhadi Moghadam B, Fereidoni M. Neuroprotective effect of menaquinone-4 (MK-4) on transient global cerebral ischemia/reperfusion injury in rat. Plos one. 2020;15(3):e0229769. DOI: 10.1371/journal.pone.0229769
29. Zhang R, Liu Y, Yan K, Chen L, Chen XR, Li P, et al. Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. J Neuroinflammation. 2013; 10(1): 871. DOI: 10.1186/1742-2094-10-106
30. Dunnett SB, Torres EM, and Annett LE. A lateralised grip strength test to evaluate unilateral nigrostriatal lesions in rats. Neurosci Lett. 1998; 246(1): 1-4. DOI: 10.1016/S0304-3940(98)00194-3
31. Gerriets T, Stolz E, Walberer M, Müller C, Kluge A, Bachmann A, et al. Noninvasive quantification of brain edema and the space-occupying effect in rat stroke models using magnetic resonance imaging. Stroke. 2004; 35(2): 566-571. DOI: 10.1161/01.STR.0000113692.38574.57
32. Liu N, Chen R, Du H, Wang J, Zhang Y, Wen J, et al. Expression of IL-10 and TNF-α in rats with cerebral infarction after transplantation with mesenchymal stem cells. Cell Mol Immunol. 2009; 6(3): 207-213. DOI: 10.1038/cmi.2009.28
33. Qiu C, Lindley A, and Jia X. Stem cell-derived extracellular vesicle therapy in ischemic brain injuries. J Stroke. 2025; 27(3): 313-328. DOI: 10.5853/jos.2025.01690
34. Song J, Zhou D, Cui L, Wu C, Jia L, Wang M, et al. Advancing stroke therapy: Innovative approaches with stem cell-derived extracellular vesicles. Cell Commun Signal. 2024; 22(1): 369. DOI: 10.1186/s12964-024-01752-1
35. Shen B, Chen J, Liu N, Hou J, and Dai Y. Therapeutic mechanisms of umbilical cord mesenchymal stem cell-derived exosomes in ischemic stroke: A transcriptomic and metabolomic study. Neural Regen Res. 2025; 21(6): 2632-2642. DOI: 10.4103/nrr.nrr-d-24-01219
36. Barbati SA, D'Amelio C, Feroleto C, Morotti M, Sarrapochiello IN, Natale F, et al. Intranasal delivery of extracellular vesicles derived from human bone marrow mesenchymal stem cells dampens neuroinflammation and ameliorates motor deficits in a mouse model of cortical stroke. Exp Neurol. 2026; 396: 115540. DOI: 10.1016/j.expneurol.2025.115540
37. Shan XQ, Luo YY, Chang J, Song JJ, Hao N, and Zhao L. Immunomodulation: The next target of mesenchymal stem cell-derived exosomes in the context of ischemic stroke. World J Stem Cells. 2023; 15(3): 52-70. DOI: 10.4252/wjsc.v15.i3.52
38. Liu J, Pan Y, Wu H, Guo Q, Fang X, Lu Y, et al. hUMSC-derived exosomes alleviate hypoxic cerebrovascular injury via AMPK/NLRP3-mediated pyroptosis suppression and mitochondrial protection. J Biomed Res. 2026; 40(3): 312-326. DOI: 10.7555/JBR.39.20250189
39. Chen N, Wang YL, Sun HF, Wang ZY, Zhang Q, Fan FY, et al. Potential regulatory effects of stem cell exosomes on inflammatory response in ischemic stroke treatment. World J Stem Cells. 2023; 15(6): 561-575. DOI: 10.4252/wjsc.v15.i6.561