Heliyon 10 (2024) e29424

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Neuroprotective effect of tanshinone IIA-modified mesenchymal stem cells in a lipopolysaccharide-induced neuroinflammation model

Jingjing Wu [a,b], Jian Chen [b], Ying Ge [b], Nanqu Huang [c,**], Yong Luo [b,*]

[a] *Medical College of Soochow University, Suzhou, Jiangsu, China*
[b] *Department of Neurology, Third Affiliated Hospital of Zunyi Medical University (The First People's Hospital of Zunyi), Zunyi, Guizhou, China*
[c] *National Drug Clinical Trial Institution, Third Affiliated Hospital of Zunyi Medical University (The First People's Hospital of Zunyi), Zunyi, Guizhou, China*

ARTICLE INFO

Keywords:
Tanshinone IIA
Mesenchymal stem cells
Neuroinflammation
Lipopolysaccharide
Alzheimer's disease

ABSTRACT

In this study, the neuroprotective potential of tanshinone IIA (TIIA)-modified mesenchymal stem cells (MSC) were investigated using a murine model of lipopolysaccharide (LPS)-induced neuroinflammation. The cognitive performance of the mice was assessed using the Y-maze and Morris water maze tests, while immunofluorescence and Western blot analyses were employed to evaluate the hippocampal expression of pertinent markers and inflammatory factors, respectively. The results from the behavioral experiments demonstrated discernible differences in learning and memory abilities between the model group and the control group ($P < 0.05$), confirming the successful induction of neuroinflammation. Both the MSC and TIIA-MSC groups exhibited enhancements in the cognitive abilities of neuroinflammatory mice, with the TIIA-MSC group demonstrating a more pronounced improvement ($P < 0.01$). Immunofluorescence analysis revealed significant activation of microglia in the model group, while the MSC and TIIA-MSC groups exhibited a reduction in hippocampal microglial activation, with the TIIA-MSC group displaying a more substantial decrease. A statistically significant difference in the expression levels of IL-1, IL-6, and TNF-α was observed between the model and control groups ($P < 0.05$), indicating that IL-1, IL-6, and TNF-α were downregulated in both the MSC and TIIA-MSC groups. Notably, the downregulatory effect was more prominent in the TIIA-MSC group ($P < 0.01$). Compared to MSC treatment alone, the administration of TIIA-modified MSC demonstrated a superior protective effect against lipopolysaccharide-induced neuroinflammation. These findings underscore the potential therapeutic efficacy of TIIA-modified MSC in mitigating neuroinflammatory responses.

1. Introduction

Neuroinflammation is an important factor in the occurrence and progression of neurodegenerative diseases and is inextricably linked to the pathogenesis of these diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease

* Corresponding author.
** Corresponding author.
E-mail addresses: hnq@zmu.edu.cn (N. Huang), luoyong@zmu.edu.cn (Y. Luo).

https://doi.org/10.1016/j.heliyon.2024.e29424
Received 20 November 2023; Received in revised form 4 April 2024; Accepted 8 April 2024
Available online 9 April 2024

[1]. The main factor of neuroinflammation is the activation of microglia, which respond quickly to imbalances in brain homeostasis caused by stress, trauma, disease, or pathology. The activation of microglia leads to the excretion of several inflammatory and cytotoxic components responsible for neuroinflammation and neurodegeneration [1]. For example, the pathogenesis of AD is characterized by neuroinflammation, which manifests as increased astrocytes, activation of microglia and increased levels of proinflammatory cytokines in the brains of patients [2–4].

The endotoxin lipopolysaccharide (LPS), which originates from bacterial outer membranes, serves as a potent inflammatory trigger [5]. Recognized as a Toll-like receptor 4 (TLR-4) ligand [6], LPS activates the production of key inflammatory cytokines, notably tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), prostaglandin E2 (PGE2), and nitric oxide (NO), in glial cells in the central nervous system (CNS) [7,8]. Consequently, LPS has become a staple in the study of neuroinflammation in murine models.

MSC, which are characterized by pluripotency, have emerged as promising candidates for AD therapy. Stem cell technology, particularly the use of MSC, is gaining traction as a potential treatment avenue due to its capacity for isolation from adult tissues, *in vitro* culture, and safe autologous or allogeneic transplantation [9,10]. The therapeutic mechanisms of bone marrow MSC (BMSC) in AD primarily involve their regulation of neuroinflammation. Despite their increasing clinical use, challenges such as immunomodulatory plasticity pose hurdles, leading to heterogeneous and unstable efficacy [11]. Modification of MSC, however, has demonstrated significantly enhanced therapeutic effects compared to their untreated counterparts [12].

Salvia miltiorrhiza, a pivotal natural medicine, tanshinone IIA (TIIA) as its principal fat-soluble constituent. Modern pharmacological investigations have elucidated the anti-inflammatory and antioxidant properties of TIIA [13,14]. Our research revealed that pretreatment of MSC with TIIA, which is rooted in traditional Chinese medicine, augments their therapeutic potential. This unique approach, which combines traditional Chinese and Western medicine theories, demonstrated the efficacy of tanshinone-induced MSC in ameliorating neuroinflammation at the cellular level [15].

Although the mechanism of the therapeutic effect of MSC has not been fully elucidated, the drug delivery pathway of MSC in animal models, such as intracranial stereotactic transplantation, is not easily accessible to the clinic [16]. In many studies on neurological diseases, caudal vein injection of MSC is a relatively common method, and we have also found support in many related studies [17–22]. Therefore, we injected bone marrow mesenchymal stem cells pretreated with TIIA into LPS-induced neuroinflammation model mice by tail vein injection, and studied its protective effect on neuroinflammation model mice.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice aged between 4 and 8 weeks were utilized in the animal experiments and were procured from the Experimental Animal Center of Zunyi Medical University. The animals were housed in a controlled environment with a relative humidity of 45%–55 %, room temperature ranging from 21 °C to 25 °C, and a 12-h light/dark cycle (8:00 a.m.-8:00 p.m.). The mice were provided unrestricted access to a free diet and water. Specifically, 4-week-old mice were subjected to primary bone marrow MSC extraction, while 8-week-old mice were used for subsequent experimental procedures.

2.2. Isolation and cultivation of MSC

For MSC isolation, 4-week-old mice were humane euthanized via cervical dislocation, followed by immersion in 75 % ethanol for 5 min. Under sterile conditions, the tibia and humerus were excised, and the periosteum and muscle tissues were meticulously separated using scissors, forceps, and sterile gauze. Subsequently, the bone marrow cavity was rinsed with basal medium (T220121d501, ORICELL) to harvest bone marrow cells. After centrifugation (1000 rpm, 5 min), the supernatant was discarded, and the cell precipitate was resuspended in complete medium (T220121d501+T220215H501, ORICELL). The bone marrow cells were evenly distributed in T25 culture bottles and cultivated in a humidified environment with 5 % carbon dioxide at 37 °C. Ethical approval for all experiments was obtained from the Ethical Committee for Animal Experiments of Zunyi Medical University (Approval no. (2019)2–231, 11 Mar 2019). Subculturing was performed when the cells reached 80 %∼90 % confluence, and cells from 3 to 5 generations were used for subsequent experiments.

2.3. MSC identification

MSC identification involved the use of anti-CD44, anti-CD90, anti-CD29, anti-CD34, and anti-CD31 antibodies (MUXMX-09011, ORICELL), which were assessed through flow cytometry. Furthermore, osteogenic, adipogenic, and chondrogenic differentiation of mouse BMSC was confirmed using induction differentiation kits for osteogenesis (MUXMX-90021, ORICELL), adipogenesis (MUXMX-90031, ORICELL), and chondrogenesis (MUXMX-90041, ORICELL).

2.4. Cell proliferation assay

In the cell proliferation assay, third-generation MSC were seeded into 96-well plates, with each well receiving 100 μL of cell suspension. The plates were incubated in a 5 % CO_2, 37 °C incubator, with the addition of 10 μL of CCK-8 solution daily. The absorbance at 450 nm was measured continuously using a microplate reader over a 6-day period. The experiment was conducted in triplicate to observe third-generation MSC proliferation.

Additionally, varying concentrations of TIIA-MSC (0, 6, 12, 24, and 48 μM) were added to cells in the logarithmic growth phase in 96-well plates. After 48 h of treatment, the TIIA was removed, 100 μL of cell suspension was added to each well, and the cells were cultured at 37 °C with 5 % CO_2. Subsequently, 10 μL of CCK-8 solution was added, and the mixture was incubated for 1 h. The absorbance at 450 nm was measured with a microplate reader. This experiment, which was conducted in triplicate, allowed us to observe the impact of TIIA on MSC proliferation and its potential toxicity.

2.5. Preparation of TIIA–MSC

TIIA (118393-10 mg, MCE) procured from MCE was dissolved in dimethyl sulfoxide (DMSO) (Solarbio, D8371, Beijing) and diluted with culture medium. After third-generation MSC were cultured with 12 μM TIIA for 48 h, the TIIA was discarded to prepare TIIA-MSC for subsequent experiments.

2.6. Mouse model of neuroinflammation induced by LPS

LPS (N1104B, sigma L2880 in parts) was obtained from Ailingfei for the induction of neuroinflammation in 8-week-old mice. The mice were divided into four groups: control (PBS), model (LPS), MSC (LPS + MSC), and TIIA-MSC (LPS + TIIA-MSC) groups. Using microsyringes and stereoscopic positioning coordinates (1.5 mm posterior to the anterior fontanelle, 1.8 mm left and right side opening, 2 mm depth), 1 μL of LPS (2 μg/μL) and PBS (control group) were injected into the left and right sides at a rate of 0.5 μL/min, and the injection was halted for 1–2 min [23,24].

2.7. Experimental protocol

Caudal intravenous injection commenced on the day following the induction of neuroinflammation. The control group (PBS) and the model group (LPS) received 0.2 mL of normal saline. The MSC in the LPS + MSC group were administered 0.2 mL of MSC (5×10^5 cells dissolved in 0.2 mL of DPBS), and those in the TIIA-MSC group (LPS + TIIA-MSC) were administered 0.2 mL of TIIA-MSC (5×10^5 cells dissolved in 0.2 mL of DPBS). Behavioral experiments were initiated on the third day, and samples were collected immediately after completion (Fig. 1).

2.8. Behavioral tests

Behavioral tests, assessing memory ability through observations in the Y maze and Morris water maze, were conducted by researchers unaware of the treatment or grouping of the mice. The tests were carried out between 08:00 a.m. and 12:00 a.m.

In the Y maze, which was placed in a quiet room, three arms with dimensions of 40 cm, 8 cm, and 15 cm, forming an angle of 120° between each arm, were utilized. On the third day after LPS injection, the mice were randomly placed in the central triangular area of the maze, facing away from a designated wall, and allowed to freely explore for 7 min [25–27]. After each mouse's test, the apparatus was cleaned to minimize interfering factors. The total number and order of arm accesses were recorded, and the spontaneous

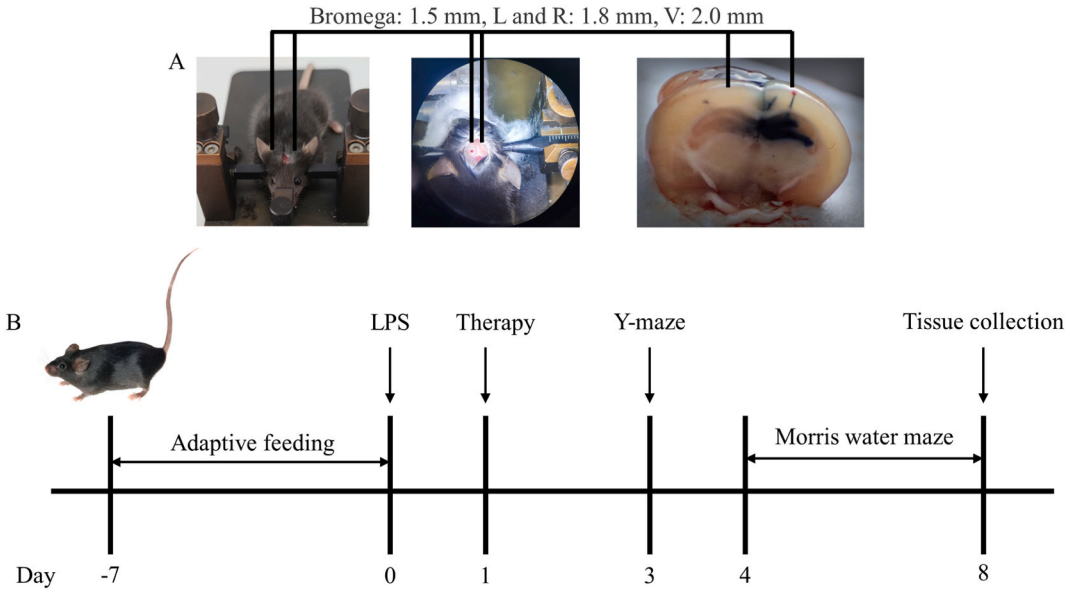

Fig. 1. Experimental operation flow chart. A. Hippocampal location in the model constructed with trypan blue. B Schematic diagram of the experimental procedures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

alternating reaction rate was calculated as (actual number of spontaneous alternating reactions/maximum possible number of spontaneous alternating reactions) × 100 %.

The Morris water maze test was performed the day after the Y maze test. The system comprised a circular pool with an automatic image acquisition and processing system. The pool, with a diameter of 160 cm, height of 50 cm, and water temperature of 21–23 °C, had a circular platform approximately 5 mm below the surface in the center of the specified target quadrant. The first four days involved directional navigation experiments conducted three times daily, lasting 60 s each. On the fifth day, the space exploration experiment was conducted with the platform removed, and mouse passage frequency and proportion of residence time were recorded within 60 s. All swim trials were performed and scored by the same operator using a computerized video tracking and recording system (TopScan Behavioral Analysis Systems, CleverSys, Inc.). The mice were allowed to rest for 10 min between trials and were subsequently allowed to dry with a heater [28].

2.9. Immunofluorescence staining

Following dewaxing and hydration, the slices were washed with PBS for 5 min, and this process was repeated three times. Subsequently, the slices were subjected to antigen retrieval in a microwave oven with citric acid antigen repair solution in a repair box. After cooling, the slices were washed with PBS, dried, and blocked with 5 % goat serum for 30 min. The membranes were incubated with the primary antibody (Iba-1, 1:500, Servicebio, GB113502) overnight, washed and dried the next day, and incubated with the fluorescent secondary antibody (CY3-labeled goat anti-rabbit IgG, 1:300, Servicebio, GB21303) corresponding to the primary antibody at room temperature for 50 min in the dark. DAPI dye solution was then added, and after washing and drying, the slides were incubated for 10 min at room temperature in the dark. Next, an anti-fluorescence quenched tablet was added to seal the slides, and photographs were taken.

2.10. Western blot

Total protein from the hippocampus was extracted using RIPA lysis buffer. The protein concentration postcleavage was determined via the BCA protein assay (Solarbio, China). Extracted protein samples were subjected to electrophoresis on 10 % or 12.5 % sodium dodecyl sulfate polyacrylamide gels, followed by transfer to PVDF membranes (Bio-Rad, USA). The PVDF membranes were subsequently blocked with 5 % bovine serum albumin. The PVDF membranes were incubated overnight at 4 °C with primary antibodies against IL-1β (ab234437, Abcam), IL-6 (A11115, ABclonal), TNF-α (60291-1-Ig, Proteintech Group), and β-actin (Cell Signaling

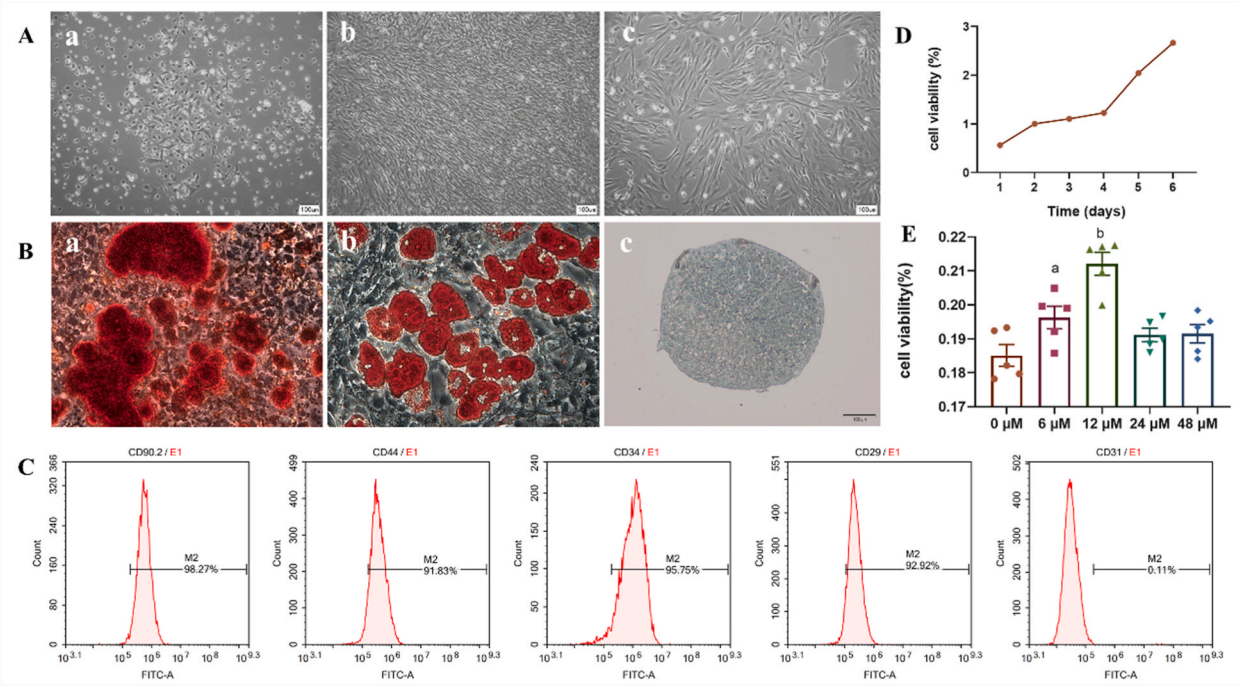

Fig. 2. Preparation and identification of MSC and TIIA-MSC. A. Morphological characteristics of MSC (× 40). B. Differentiation and identification of MSC. a. Alizarin red staining (× 100), b. Oil red O staining (× 100), c. Alysin blue staining (100). C. Analysis of MSC phenotypes. D. Expression of CD90 (98.27 %), CD44 (91.83 %), CD34 (95.75 %), CD31 (0.11 %), and CD29 (92.92 %). Growth curve of MSC in the P3 generation. E. Effect of TIIA concentration on the proliferation of MSC. Compared with 0 μM, $^{a}P < 0.05$, $^{b}P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Technology, USA; 1:1000). After 12–16 h of incubation with primary antibodies, the PVDF membranes were washed with HRP-conjugated secondary rabbit or anti-mouse antibodies (Beyotime, China, 1:10000) and incubated for 1 h at room temperature. Subsequently, the bands were visualized using an enhanced chemiluminescence (ECL) detection kit (Beyotime, China).

2.11. Statistical analysis

All the data were statistically analyzed using SPSS 22.0. Unless otherwise specified, experiments were conducted with three samples and repeated a minimum of three times. The data are presented as the means ± SDs and were subjected to one-way ANOVA and further analyzed using Tukey's honest significance difference test. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Characteristics of MSC

Primary-to third-generation BMSC exhibited rapid proliferation (Fig. 2-A, D). Flow cytometry identified the third generation of BMSC, confirming their differentiation into osteoblasts, chondrocytes, and lipids, as illustrated in Fig. 2-B. Immunophenotype analysis revealed consistent expression of CD90, CD44, CD29, and CD34 across all samples, with the absence of CD31 (Fig. 2-C).

3.2. Intervention of TIIA in MSC

After MSC were subjected to 48 h of TIIA interference, the TIIA was removed, and its impact on MSC proliferation was assessed. The results indicated a noticeable increase in MSC proliferation at TIIA concentrations of 6 μM and 12 μM compared to that of the untreated group, with the optimal effect observed at 12 μM. However, concentrations exceeding 24 μM resulted in decreased proliferation and activity of MSC (Fig. 2-E).

3.3. TIIA-MSC ameliorate learning and memory impairment in mice with LPS-induced neuroinflammation

The results of the Y-maze test indicated that the spontaneous alternation rate in the normal group was greater than that in the model group. Conversely, both the MSC group and the TIIA-MSC group demonstrated significantly greater spontaneous alternations than did the model group, with the TIIA-MSC group exhibiting a more pronounced difference than did the MSC group (Fig. 3-A).

In the water maze experiment, starting from the initial day of training, the model group exhibited distinct differences in recognizing or remembering the platform compared to the other groups. Over subsequent training sessions, the time taken by the model group to

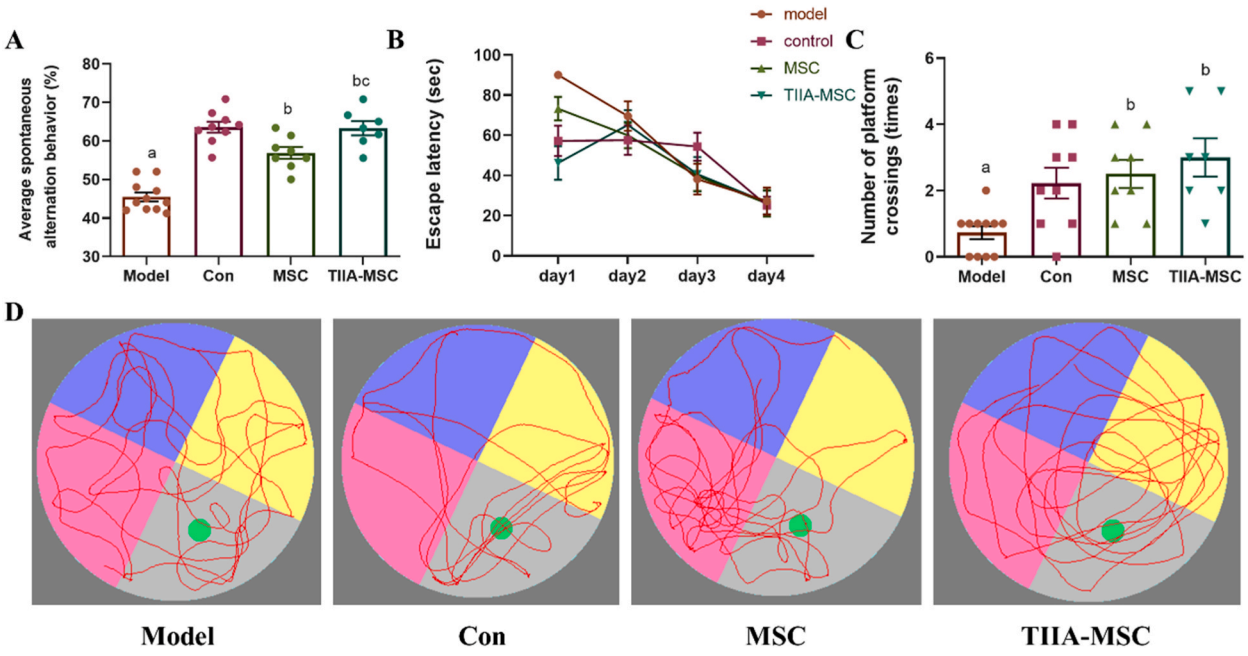

Fig. 3. Behavioral tests through the Y-maze and Morris water maze. A. Rate of spontaneous alternation in the Y-maze. B. The first four days of escape incubation in a water maze. C. The number of platform crossings in the target quadrant on Day 5 of the water maze. D. A probe test in a water maze recorded the swimming paths of the mice. Compared with the control group, $^{a}P < 0.05$ indicated successful modeling. Compared with the model group, $^{b}P < 0.05$. Compared with the MSC group, for which $^{c}P < 0.05$, the TIIA-MSC group had better results.

locate the platform was consistently longer than that taken by the other groups, albeit with a decreasing trend, which is indicative of the learning process. The control group exhibited the shortest time spent finding the platform during training. Both the MSC group and the TIIA-MSC group spent less time finding the platform than did the model group, especially on the last day of the space exploration experiment. The number of crossings over the original platform in the target quadrant was the lowest in the model group and the greatest in the control group. Both the MSC and TIIA-MSC groups outperformed the model group, with the TIIA-MSC group exhibiting a more significant difference (Fig. 3-B, C, D).

3.4. TIIA-MSC inhibit microglial activation in mice with LPS-induced neuroinflammation

Immunofluorescence staining revealed significant activation of microglia in the hippocampus of the model group. In both the MSC group and the TIIA-MSC group, the activation of microglia was reduced, with the TIIA-MSC group showing a more pronounced reduction (Fig. 4).

3.5. TIIA-MSC alleviate inflammatory responses in LPS-induced neuroinflammatory mice

Compared with those in the control group, the levels of the neuroinflammatory cytokines IL-1, IL-6, and TNF-α were significantly increased in the model group. The expression of IL-1, IL-6, and TNF-α decreased in both the MSC group and the TIIA-MSC group, with the TIIA-MSC group demonstrating a more substantial decrease in expression (Fig. 5).

4. Discussion

Neuroinflammation is triggered by an imbalance of immune cells in the CNS, of which microglia and astrocytes are key cell types. Neuroinflammation is the result of CNS damage, infection, toxicity, or autoimmunity. Microglia, a kind of glial cells, are at the forefront of the CNS, the immune system's defense system. Unbalanced and unstable microglia and astrocytes are different types of glial cells that impair the clearance of protein aggregates (amyloid-beta plaques) in AD [29,30]. Neuroinflammation usually occurs before the appearance of histopathological or pathological features of AD [31]. The activation of microglia may lead to the production of proinflammatory cytokines, ultimately leading to neuronal damage and resulting in learning and memory impairments [4]. Therefore, we studied the effect of TIIA-MSC on improving neuroinflammation in a mouse model of neuroinflammation, which will be conducive to the later application of TIIA-MSC in treating diseases such as AD.

Stem cells are undifferentiated cells that can differentiate into different types of cells and tissues from individual cells that self-

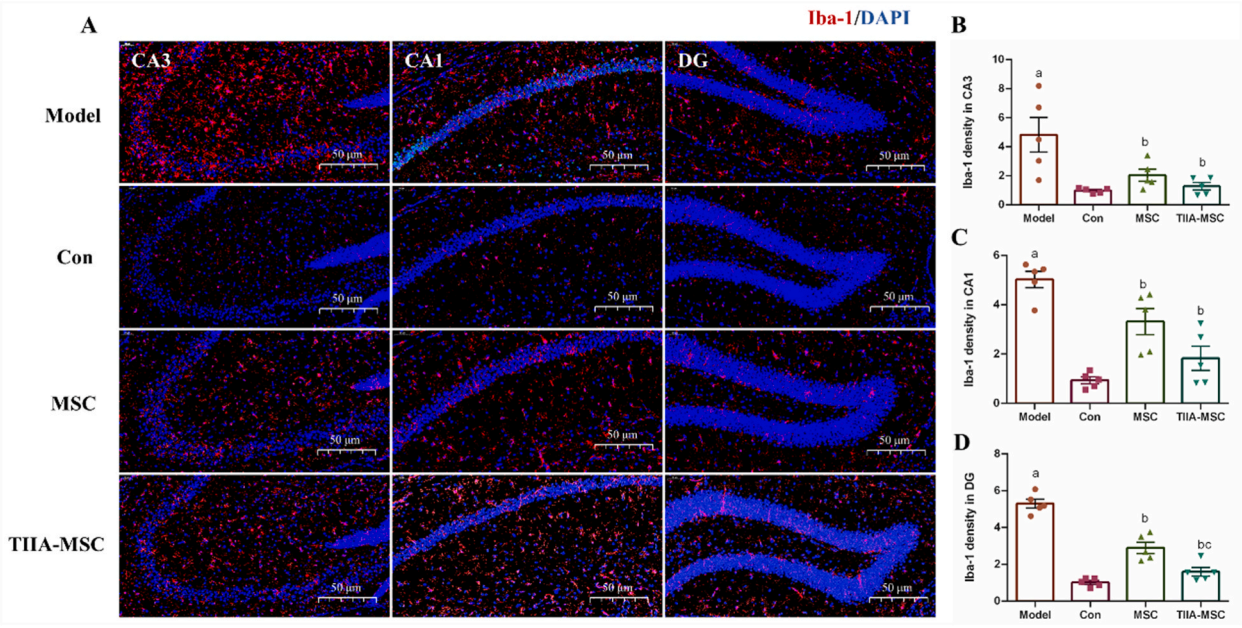

Fig. 4. TIIA-MSC inhibit hippocampal microglial activation in mice with LPS-induced neuroinflammation, as determined by immunofluorescence. A. The expression of Iba-1 (red) and DAPI (blue) in the hippocampus was analyzed by immunofluorescence. B. Iba-1 expression in the hippocampal CA3 region. C. Iba-1 expression in the hippocampal CA1 region. D. Iba-1 expression in the hippocampal DG. The expression of the control group was normalized as a relative change. Compared with the control group, $^{a}P < 0.05$ indicated successful modeling. Compared with the model group, $^{b}P < 0.05$. Compared with the MSC group, for which $^{c}P < 0.05$, the TIIA-MSC group had better results. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

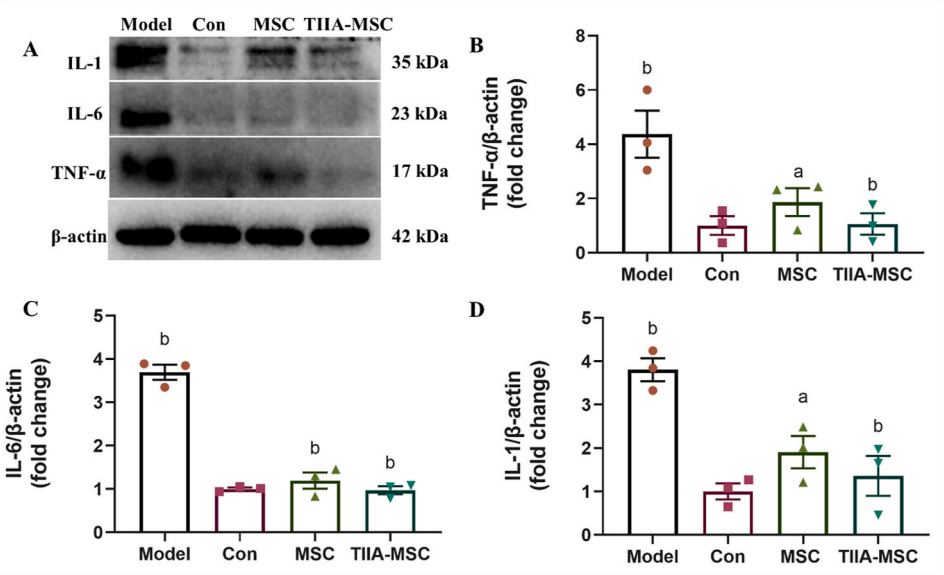

Fig. 5. TIIA-MSC inhibited the expression of inflammatory cytokines in the hippocampus. A. WB analysis of IL-1, IL-6, and TNF-α levels. B. The protein levels of IL-1 were detected by WB. C. The protein levels of IL-6 were detected by WB. D. The protein levels of TNF-α were detected by WB. The data are presented as the means ± SDs of three separate experiments. Compared to the control group, $^{a}P < 0.05$; compared to the model group, $^{b}P < 0.01$.

renew to proliferate. MSC are adult stem cells that can be obtained from various tissues, including bone marrow, adipose tissue, bone, cord blood and peripheral blood [32]. MSC have important functions *in vivo*; they can migrate to the injured site and participate in the repair process [33]. Compared to other stem cells, MSC have greater differentiation potential because they can differentiate into nerve cells, bone cells, chondrocytes, or fat cells when stimulated by certain growth factors. In addition, BMSC have a low risk of differentiating into cancer cells and are rarely immunogenic [34]. Therefore, BMSC transplantation is also a promising treatment. In this study, primary mouse bone marrow mesenchymal stem cells were extracted and identified by flow flow and differentiation. In the flow identification, CD90, CD44, CD29 and CD34 were expressed positively in all samples, while CD31 was expressed negatively. This is indeed inconsistent with some studies, but we have also consulted domestic and foreign literature, and the expression of CD34 is indeed controversial. In some studies, the expression of CD34 is positive [35]. Lee et al. [36] isolated a group of muscle-derived $CD34^{+}$ stem cells that promote muscle regeneration and bone healing. Garcia-Pacheco et al. [37] found that CD34-positive human decidual stromal cells are related to bone marrow stromal precursors. In addition, the expression of CD34 in bone marrow mesenchymal stem cells has also been reported, and it has been found that it is gradually lost after culture *in vitro* [38,39]. Maybe different cells are being lost at different rates, so that could be the reason. Of course, in our follow-up studies, we will also pay attention to this problem, and perhaps add more phenotypic identification to verify our results.

MSC have remarkable plasticity. Studies have shown that various anti-inflammatory effects of MSC may occur in complex inflammatory environments, resulting in unstable immunomodulatory effects. When the immune system is not activated, MSC can exert proinflammatory effects, but when the immune system is stimulated to avoid self-attack, MSC can exert anti-inflammatory effects [40]. In this study, MSC showed significant anti-inflammatory effects, indicating that LPS can induce an inflammatory response and activate the immune system in mice. However, the efficacy of MSC transplantation is poor, and the limited survival rate is the main obstacle to clinical application. Although many studies have focused on improving the stemness and differentiation of MSC *in vivo* and *in vitro*, MSC-based regenerative medicine still lacks effective and safe drugs [41]. In addition, long-term *in vitro* culture may lead to aging of MSC, reduced cell self-renewal, increased cell senescence, increased apoptosis, and upregulated premature differentiation, thus reducing the therapeutic effect of MSC *in vivo* [41]. The transformation of MSC is a promising research direction.

TIIA, considered a fundamental element in traditional Chinese medicine, is a pivotal lipophilic diterpenoid compound extracted from *Salvia miltiorrhiza*. A wealth of experimental and clinical studies attests to the multifaceted therapeutic potential of TIIA, demonstrating its efficacy in preventing or decelerating the progression of various ailments, including cardiovascular diseases, cancer, cerebrovascular diseases, and AD [30,42]. Intriguingly, an expanding body of research supports the notion that TIIA has a protective effect against neuroinflammation [30,43,44]; this underscores its significance not only as a traditional remedy but also as a promising avenue for addressing modern health care challenges. The exploration of the mechanisms and applications of TIIA continues to progress, fostering a deeper understanding of its potential in combating diseases caused by inflammatory components, particularly in the field of neurological health.

In this study, a notable increase in MSC proliferation was observed after a 48-h coincubation with TIIA, emphasizing the potential synergistic effects of these components. When TIIA-modified MSC were transplanted into a mouse model of neuroinflammation-induced AD, a significant improvement in the learning and memory abilities of the mice was evident compared to those in the

MSC-only group. To address a crucial therapeutic goal in AD, we investigated whether TIIA-primed MSC could effectively inhibit microglial activation and attenuate neuroinflammatory responses *in vivo*. Immunofluorescence staining revealed a notable reduction in the number of activated Iba-1-positive microglia in both the MSC and TIIA-MSC groups, with the TIIA-MSC group displaying a more pronounced inhibitory effect. Additionally, Western blot results indicated that both groups effectively reduced the expression of the neuroinflammatory cytokines IL-1, IL-6, and TNF-α. Notably, the TIIA-MSC group exhibited a more significant regulatory effect overall.

The results obtained in this study not only strengthen the therapeutic potential of MSC in neuroinflammation-related diseases but also provide a new perspective for expanding the wide application of MSC. However, the precise mechanisms underlying the TIIA-mediated modification of the anti-inflammatory effects of MSC warrant further research and in-depth discussion. Moreover, future investigations should focus on positive regulatory inflammatory factors to verify relevant negative regulatory inflammatory factors and explore potential underlying mechanisms. This modified approach to MSC provides valuable insights into the development of treatments for neuroinflammation-related diseases. At the same time, although many previous studies have confirmed that MSC injected through the tail vein can enter brain tissue via the blood-brain barrier [19,45], we can still continue relevant research work in future studies. In order to better understand the mechanism of action of MSC, we plan to use Dil labeling technology to label MSC in subsequent studies and then inject them into mice via the tail vein. The goal of this step is to more precisely track the migration and distribution of MSC in the body and further confirm whether they are able to cross the blood-brain barrier, thus entering the central nervous system and playing a role in anti-neuroinflammation.

5. Conclusion

The results of this study highlight the substantial anti-inflammatory and neuroprotective effects of TIIA-MSC and support their potential efficacy as a promising therapeutic pathway for the treatment of neuroinflammation-related diseases. Importantly, these data provide new perspectives and innovative approaches for the application of modified MSC. The results of this study provide valuable insights into the treatment and development prospects of neuroinflammation-related diseases, opening new horizons for effective interventions for neuroinflammatory diseases.

Funding

This work was supported by the National Natural Science Foundation of China (82160858), the Science and Technology Department of Guizhou Province (ZK [2021]-570, ZK [2024]-680), the Zunyi Science and Technology Bureau (HZ-2022-45), and the Funds of The First People's Hospital of Zunyi for Research and Experimental Development (R&D 2020–06).

Ethics approval

All experiments were approved by the Animal Experiment Ethics Committee of Zunyi Medical University (No.: (2019)2–231, March 11, 2019).

Data availability statement

The raw measurements are available in the Supplemental Files.

CRediT authorship contribution statement

Jingjing Wu: Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation. **Jian Chen:** Data curation. **Ying Ge:** Project administration. **Nanqu Huang:** Writing – review & editing, Funding acquisition. **Yong Luo:** Supervision, Funding acquisition, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29424.

References

[1] D.M. Teleanu, A.G. Niculescu, I.I. Lungu, C.I. Radu, O. Vladâcenco, E. Roza, et al., An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases, Int. J. Mol. Sci. 23 (2022).
[2] A. Serrano-Pozo, M.L. Mielke, T. Gómez-Isla, R.A. Betensky, J.H. Growdon, M.P. Frosch, et al., Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease, Am. J. Pathol. 179 (2011) 1373–1384.
[3] M.T. Heneka, M.P. Kummer, E. Latz, Innate immune activation in neurodegenerative disease, Nat. Rev. Immunol. 14 (2014) 463–477.
[4] M. Losurdo, M. Pedrazzoli, C. D'Agostino, C.A. Elia, F. Massenzio, E. Lonati, et al., Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease, Stem Cells Transl Med 9 (2020) 1068–1084.
[5] M.N. Catorce, G. Gevorkian, LPS-Induced murine neuroinflammation model: main features and suitability for pre-clinical assessment of nutraceuticals, Curr. Neuropharmacol. 14 (2016) 155–164.
[6] B. Beutler, Tlr4: central component of the sole mammalian LPS sensor, Curr. Opin. Immunol. 12 (2000) 20–26.
[7] S. Lehnardt, C. Lachance, S. Patrizi, S. Lefebvre, P.L. Follett, F.E. Jensen, et al., The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS, J. Neurosci. 22 (2002) 2478–2486.
[8] R.E. Mrak, W.S. Griffin, Glia and their cytokines in progression of neurodegeneration, Neurobiol. Aging 26 (2005) 349–354.
[9] Y. Torrente, E. Polli, Mesenchymal stem cell transplantation for neurodegenerative diseases, Cell Transplant. 17 (2008) 1103–1113.
[10] V.M. Rodríguez-Pardo, J.P. Vernot, Mesenchymal stem cells promote a primitive phenotype CD34+c-kit+ in human cord blood-derived hematopoietic stem cells during ex vivo expansion, Cell. Mol. Biol. Lett. 18 (2013) 11–33.
[11] C. Zeltz, I. Primac, P. Erusappan, J. Alam, A. Noel, D. Gullberg, Cancer-associated fibroblasts in desmoplastic tumors: emerging role of integrins, Semin. Cancer Biol. 62 (2020) 166–181.
[12] S.O. Sadatpoor, Z. Salehi, D. Rahban, A. Salimi, Manipulated mesenchymal stem cells applications in neurodegenerative diseases, Int J Stem Cells. 13 (2020) 24–45.
[13] R. Guo, L. Li, J. Su, S. Li, S.E. Duncan, Z. Liu, et al., Pharmacological activity and mechanism of tanshinone IIA in related diseases, Drug Des. Dev. Ther. 14 (2020) 4735–4748.
[14] H. He, T. Yang, F. Li, L. Zhang, X. Ling, A novel study on the immunomodulatory effect of umbilical cord derived mesenchymal stem cells pretreated with traditional Chinese medicine Asarinin, Int. Immunopharm. 100 (2021) 108054.
[15] N. Huang, J. Huang, F. Feng, Z. Ba, Y. Li, Y. Luo, Tanshinone IIA-incubated mesenchymal stem cells inhibit lipopolysaccharide-induced inflammation of N9 cells through TREM2 signaling pathway, Stem Cell. Int. 2022 (2022) 9977610.
[16] S. Reyes, N. Tajiri, C.V. Borlongan, Developments in intracerebral stem cell grafts, Expert Rev. Neurother. 15 (2015) 381–393.
[17] A.F. Neves, C. Camargo, C. Premer, J.M. Hare, B.S. Baumel, M. Pinto, Intravenous administration of mesenchymal stem cells reduces Tau phosphorylation and inflammation in the 3xTg-AD mouse model of Alzheimer's disease, Exp. Neurol. 341 (2021) 113706.
[18] T. Kanamaru, N. Kamimura, T. Yokota, K. Nishimaki, K. Iuchi, H. Lee, et al., Intravenous transplantation of bone marrow-derived mononuclear cells prevents memory impairment in transgenic mouse models of Alzheimer's disease, Brain Res. 1605 (2015) 49–58.
[19] O. Lykhmus, L. Koval, L. Voytenko, K. Uspenska, S. Komisarenko, O. Deryabina, et al., Intravenously injected mesenchymal stem cells penetrate the brain and treat inflammation-induced brain damage and memory impairment in mice, Front. Pharmacol. 10 (2019) 355.
[20] Y. Naaldijk, C. Jäger, C. Fabian, C. Leovsky, A. Blüher, L. Rudolph, et al., Effect of systemic transplantation of bone marrow-derived mesenchymal stem cells on neuropathology markers in APP/PS1 Alzheimer mice, Neuropathol. Appl. Neurobiol. 43 (2017) 299–314.
[21] J.Y. Shin, H.J. Park, H.N. Kim, S.H. Oh, J.S. Bae, H.J. Ha, et al., Mesenchymal stem cells enhance autophagy and increase β-amyloid clearance in Alzheimer disease models, Autophagy 10 (2014) 32–44.
[22] C. Qin, Y. Lu, K. Wang, L. Bai, G. Shi, Y. Huang, et al., Transplantation of bone marrow mesenchymal stem cells improves cognitive deficits and alleviates neuropathology in animal models of Alzheimer's disease: a meta-analytic review on potential mechanisms, Transl. Neurodegener. 9 (2020) 20.
[23] D. Chugh, P. Nilsson, S.A. Afjei, A. Bakochi, C.T. Ekdahl, Brain inflammation induces post-synaptic changes during early synapse formation in adult-born hippocampal neurons, Exp. Neurol. 250 (2013) 176–188.
[24] E. Domínguez-Rivas, E. Ávila-Muñoz, S.W. Schwarzacher, A. Zepeda, Adult hippocampal neurogenesis in the context of lipopolysaccharide-induced neuroinflammation: a molecular, cellular and behavioral review, Brain Behav. Immun. 97 (2021) 286–302.
[25] K. Sun, P. Yang, R. Zhao, Y. Bai, Z. Guo, Matrine attenuates D-Galactose-induced aging-related behavior in mice via inhibition of cellular senescence and oxidative stress, Oxid. Med. Cell. Longev. 2018 (2018) 7108604.
[26] K. Yoshizaki, M. Asai, T. Hara, High-fat diet enhances working memory in the Y-maze test in Male C57BL/6J mice with less anxiety in the elevated plus maze test, Nutrients 12 (2020).
[27] A.L. Dinel, C. Lucas, D. Guillemet, S. Layé, V. Pallet, C. Joffre, Chronic supplementation with a Mix of Salvia officinalis and Salvia lavandulaefolia improves Morris water maze learning in normal adult C57Bl/6J mice, Nutrients 12 (2020).
[28] S. Qiu, J.P. Palavicini, J. Wang, N.S. Gonzalez, S. He, E. Dustin, et al., Adult-onset CNS myelin sulfatide deficiency is sufficient to cause Alzheimer's disease-like neuroinflammation and cognitive impairment, Mol. Neurodegener. 16 (2021) 64.
[29] S. Yu, Y.P. Liu, H.L. Liu, J. Li, Y. Xiang, Y.H. Liu, et al., Serum protein-based profiles as novel biomarkers for the diagnosis of Alzheimer's disease, Mol. Neurobiol. 55 (2018) 3999–4008.
[30] F. Maione, M. Piccolo, S. De Vita, M.G. Chini, C. Cristiano, C. De Caro, et al., Down regulation of pro-inflammatory pathways by tanshinone IIA and cryptotanshinone in a non-genetic mouse model of Alzheimer's disease, Pharmacol. Res. 129 (2018) 482–490.
[31] D. Singh, Astrocytic and microglial cells as the modulators of neuroinflammation in Alzheimer's disease, J. Neuroinflammation 19 (2022) 206.
[32] Q. Yin, Introduction to the special issue on stem cells and regenerative medicine, Eur. J. Histochem. 64 (2020).
[33] N.P. Staff, D.T. Jones, W. Singer, Mesenchymal stromal cell therapies for neurodegenerative diseases, Mayo Clin. Proc. 94 (2019) 892–905.
[34] J. Kim, Y. Lee, S. Lee, K. Kim, M. Song, J. Lee, Mesenchymal stem cell therapy and Alzheimer's disease: current status and future perspectives, J Alzheimers Dis 77 (2020) 1–14.
[35] C. Viswanathan, R. Kulkarni, A. Bopardikar, S. Ramdasi, Significance of CD34 negative hematopoietic stem cells and CD34 positive mesenchymal stem cells - a valuable dimension to the current understanding, Curr. Stem Cell Res. Ther. 12 (2017) 476–483.
[36] J.Y. Lee, Z. Qu-Petersen, B. Cao, S. Kimura, R. Jankowski, J. Cummins, et al., Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing, J. Cell Biol. 150 (2000) 1085–1100.
[37] J.M. García-Pacheco, C. Oliver, M. Kimatrai, F.J. Blanco, E.G. Olivares, Human decidual stromal cells express CD34 and STRO-1 and are related to bone marrow stromal precursors, Mol. Hum. Reprod. 7 (2001) 1151–1157.
[38] N. Quirici, D. Soligo, P. Bossolasco, F. Servida, C. Lumini, G.L. Deliliers, Isolation of bone marrow mesenchymal stem cells by anti-nerve growth factor receptor antibodies, Exp. Hematol. 30 (2002) 783–791.
[39] P.J. Simmons, B. Torok-Storb, CD34 expression by stromal precursors in normal human adult bone marrow, Blood 78 (1991) 2848–2853.
[40] W. Jiang, J. Xu, Immune modulation by mesenchymal stem cells, Cell Prolif. 53 (2020) e12712.
[41] C. Hu, L. Li, The application of resveratrol to mesenchymal stromal cell-based regenerative medicine, Stem Cell Res. Ther. 10 (2019) 307.
[42] J.G. Wang, S.C. Bondy, L. Zhou, F.Z. Yang, Z.G. Ding, Y. Hu, et al., Protective effect of Tanshinone IIA against infarct size and increased HMGB1, NFκB, GFAP and apoptosis consequent to transient middle cerebral artery occlusion, Neurochem. Res. 39 (2014) 295–304.

[43] Y. He, J.B. Ruganzu, C. Lin, B. Ding, Q. Zheng, X. Wu, et al., Tanshinone IIA ameliorates cognitive deficits by inhibiting endoplasmic reticulum stress-induced apoptosis in APP/PS1 transgenic mice, Neurochem. Int. 133 (2020) 104610.
[44] W. Yang, J. Zhang, L. Shi, S. Ji, X. Yang, W. Zhai, et al., Protective effects of tanshinone IIA on SH-SY5Y cells against oAβ(1-42)-induced apoptosis due to prevention of endoplasmic reticulum stress, Int. J. Biochem. Cell Biol. 107 (2019) 82–91.
[45] Y. Yang, Q. Liu, S. Deng, Q. Shao, L. Peng, Y. Ling, et al., Human umbilical cord derived mesenchymal stem cells overexpressing HO-1 attenuate neural injury and enhance functional recovery by inhibiting inflammation in stroke mice, CNS Neurosci. Ther. 30 (2024) e14412.