Research Article

Mesenchymal Stem Cells Improve Ischemic Stroke Injury by Anti-Inflammatory Properties in Rat Model of Middle Cerebral Artery Occlusion

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Abstract

Background: Ischemic stroke is a major cause of permanent disability and inflammation has a prominent role in stroke pathology. Stem cell therapy is a new approach for stroke treatment. Mesenchymal stem cells (MSCs) are appropriate for this approach due to neuroprotective and immunomodulatory effects.

Objectives: In this experimental study, the neuroprotective effects of mesenchymal stem cells (MSCs) on brain injury after transient middle cerebral artery occlusion (tMCAO) in rats was investigated with emphasis on inflammatory factors.

Methods: Mesenchymal Stem Cells were isolated from bone marrow of rats and expanded by cell culture. Thirty-six male Wistar rats were randomly selected and divided to 6 groups. The MCAO model was performed in 4 groups with 24 and 72 hours of reperfusion. A single infusion of 2×10^6 MSCs was transplanted in one of the 24-hour and 72-hour groups and others received saline. In the sham groups, surgery was done without MCAO. Behavioral tests were evaluated and infarct volume was measured by staining of brain sections. Serum levels of Interleukin (IL)1β and Tumor necrosis factor (TNF)α were measured by the enzyme linked immunosorbent assay (ELISA). Relative expression of Interleukin (IL)1β, tumor necrotizing factor (TNF)α, and IL6 genes were assessed in penumbra of the ischemic region using real time polymerase chain reaction (PCR).

Results: The study results indicated that total behavioral scores were increased 72 hours after MSC transplantation (14.5 $\pm$ 2.0, P < 0.01). Moreover, MSCs decreased the infarct volume both 24 hours (18.82 $\pm$ 1.58, P < 0.01) and 72 hours (14.4 $\pm$ 1.53, P < 0.05) after MCAO. Serum levels of IL-1β and TNFα were increased after MCAO, yet MSCs transplantation decreased IL-1β (368.3 $\pm$ 109.5, P < 0.001) and TNFα (126.9 $\pm$ 38.6, P < 0.01) compared to saline. Also, relative gene expression of IL1β, TNFα, and IL6 was decreased by MSCs transplantation (P < 0.05).

Conclusions: The MSCs had a neuroprotective effect in ischemic stroke via modulation of inflammatory response, and serum levels of IL1β and TNFα could be used as markers for evaluating anti-inflammatory effects of MSCs.

Keywords: Mesenchymal Stem Cell, Ischemic Stroke, Inflammation, MCAO

1. Background

Ischemic stroke is known as a major cause of mortality and continual disability worldwide. Despite the advancement in biology and pathology of stroke, effective treatment is not available. Ischemic stroke is characterized by cerebral artery blockage and abrupt restriction of blood supply to an area of the brain tissue (1). Lack of oxygen and glucose in the core of ischemic region leads to neural cells necrosis, while surrounding cells are compromised. Any treatment that can save these cells is effective in reducing the infarct zone and complications of ischemic stroke. Transient ischemia and subsequent reperfusion trigger oxidative damage, excitotoxicity, and inflammatory response

(2). The entrance of cerebral resident macrophage, microglia (3, 4), and dendritic cells (5) in to injured brain tissue, and the release of cytokines, chemokines and infiltration of peripheral blood monocyte, granulocyte and lymphocytes (6) exacerbated the inflammation and subsequent expansion of ischemic lesions (7). It has been reported that suppression of inflammation in the early stage of ischemic stroke could be reduced in the infarct zone and neural injures (8). Therefore, stem cell therapy seems to be a new horizon in ischemic stroke recovery. This study examined the blood levels of inflammatory factors after brain ischemia and MSCs transplantation and suggested these factors as markers of stroke assessment and therapeutic procedures.

Mesenchymal stem cells (MSCs) are multipotent adult stem cells with neuroprotective effects ([9]). Although the exact mechanisms of this protection are unknown, modulation of inflammatory response ([10], [11]), decrease of macrophages and neutrophils infiltration ([12]), activations of microglia and macrophages and suppression of T and B cells maturation ([13], [14]), has been mention in experimental studies as probable mechanisms. Moreover, ability of migration to damaged tissue, release of growth factor and cytokine and trans-diffrentiation into neurons and glial cells are some properties that make MSCs a good choice for cell therapy of ischemic stroke ([15]). The current study investigated the effects of bone marrow-derived mesenchymal stem cells on motor and sensory defects recovery, infarct size, gene expression of pro-inflammatory cytokines in brain tissue and serum levels of these cytokines after MCAO in rat.

2. Methods

This study was performed at the anatomical sciences research center, Kashan University of Medical Sciences (KAUMS), Kashan, Iran during year 2016.

2.1. Animals

Male Wistar rats aged 8 to 10 weeks old and weighting 210 to 250 g were prepared at Kashan University of medical sciences lab animal center. Rats were bred and maintained in a pathogen-free environment at a temperature of $20 \pm 2°C$ and had free access to food and water under a 12-hour light/dark cycle throughout the experiment. Animal care and all of experimental procedures were confirmed by the ethics committee of KAUMS. The code and date of ethical approval was 2012.06.01 (p/29/5/1/932).

2.2. Isolation, Expansion and Characterization of Mesenchymal Stem Cells

Mesenchymal stem cells were derived from bone marrow of rats. Once male Wistar rats (4 to 6 week old) were sacrificed, tibia and femoral bones were removed under aseptic conditions and bone marrow was flushed out using a 5-mL syringe and 23-gauge needle with cell culture media. The cell suspension was centrifuged at 360 g for 5 minutes. Sedimentary cells were suspended in high-glucose DMEM (Invitrogen; USA) that was supplemented with 10% (v/v) fetal bovine serum (Invitrogen; USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma; Germany), and seeded in 75 cm² cell culture flasks (Orange Scientific, Belgium). Flasks were placed in an incubator (Memmert; Germany) with 5% CO2 and 85% humidity at 37°C temperature. After 24 hours, media and non-attached cells were discarded and replaced with fresh media. Adherent cells were

allowed to expand in the cell culture flask. The medium was exchanged once every 2 days.

Bone marrow-derived MSCs were identified by potential differentiation to adipocytes and osteocytes. For demonstrating of this capability, 5000/cm² cells were seeded in 6-well plates and adipogenic differentiation medium was added that contained low glucose DMEM (Invitrogen; USA) supplemented with FBS and penicillin/streptomycin (same as the growth media), plus 50 μg/mL of ascorbic acid- 2 phosphate, 100 nM dexamethasone, and 50 μg/mL indomethacin (all from Sigma; Germany). The media was exchanged half-and-half every 2 days. After 21 days, the cells were fixed and stained by Oil red O (Sigma-Aldrich) and investigated for fat vacuoles and adipocyte cells. Similarly, osteogenic differentiation medium consisting of the growth media plus 10 mM glycerol-2-phosphate, 50 μg/mL ascorbic acid- 2- phosphate, and 100 nM dexamethasone (all from Sigma-Aldrich) were added to plated cells. The media was exchanged half-and-half every 2 days, and cell variation was evaluated under the microscope. After 21 days, cells were fixed and stained by Alizarin Red (Sigma-Aldrich) and checked for osteocyte and calcium sediments in the cytoplasm and intra cellular matrix.

Bone marrow-derived cells were characterized with surface CD markers, which were checked by flow cytometry. In brief, the cell suspension was washed twice with PBS. For direct assays, one million cells were incubated with PE-conjugated anti-CD34 antibody (Abcam, USA), anti-CD45 antibody (Santa Cruz Biotechnology_ USA), anti-CD44 antibody (Abcam, USA), anti-CD73 antibody (SH-3) (Cell Signaling Technology, USA), anti-CD90 antibody (Abcam, USA), and anti-CD105 (SH- 2) antibody (Abcam, USA) at 4°C for 30 minutes, in the dark and then washed twice with PBS. The cells were analyzed by cytometric analysis using the BD FACS Calibur flow cytometer (BD Biosciences, USA) and interpreted with Flowjo 7.6.1 software; MSCs in passages 3 to 5 were used for cell therapy.

2.3. Transient Middle Cerebral Artery Occlusion

An experimental model of focal ischemia in the rat was performed using transient middle cerebral artery occlusion (tMCAO) as previously described with few modifications ([16], [17]). In brief, the rats were anesthetized by inhalation of 4% isoflurane (Piramal Critical Care; USA) via a face mask and maintained under anesthesia with 2% isoflurane throughout the surgery. Rats were reposed on a warm pad (Narco Bio System; USA) to maintain their body temperature at 37 $\pm$ 0.5°C during the operation. Cerebral Blood Flow (CBF) was measured using a Laser-Doppler flowmeter (Moor Instruments VMS-LDF2, UK). Probes of the Laser-Doppler were fixed on the drill-thinned skull, in position,

4 mm lateral and 2 mm posterior to the bregma, on both ipsi/contra lateral hemisphere and basic CBF was recorded. A middle longitudinal incision on the neck was performed and left common carotid artery (CCA) was exposed. Next, a commercially available catheter (Doc Cole, USA) was inserted in the lumen of the CCA and propelled to the Internal Carotid Artery (ICA), until it reached to a stay or an abrupt fall in the CBF occurred. At this point, the catheter occluded the entrance of the MCA, and the catheter was fixed by a clamp and left to rest for 60 minutes. The CBF was monitored during the occlusion. Then the catheter was removed and CCA was ligated permanently. The MCA was perfused again through the cerebral arterial circle (Wilis loop), and CBF returned to almost the previous amount. Three hours after reperfusion, 2×10^6 MSCs in 0.5 mL saline buffer were infused via the tail vein in both the 24-hour and 72-hour MSC groups, and the rats in the control groups only received 0.5 mL of saline.

2.4. Behavioral Assessment

Garsia motor and sensory behavioral tests were performed with a few changes as described (18), exerted to assess post-ischemic neurological deficits. The following 6 tests were performed before ischemic stroke and 24 or 72 hours after reperfusion by 2 blinded readers.

Spontaneous activity: Rats were placed in the middle of a 60 × 90-cm sized cage within an unknown environment for 3 minutes. Scoring was done as follows 0 = rats not moving; 1 = rats moved hardly but not rising up in the cage; 2 = rats moving around in the cage waveringly yet not approaching the walls, nevertheless ultimately going up in the cage; 3 = rats moving around, seeking the environment and approaching three walls of the cage.

Forepaw outstretching: Rats were lifted by the tail and the synchrony of the left and right forelimbs outstretching was checked. Scores: 0 = right forelimb entirely constricted, 1 = right side moves faintly; 2 = outstretching on right side was less than the left side; 3 = equal outstretching in both forelimbs.

Ability to climb: Rats were placed on a 2-cm step space stairwell. Normally rats use all four limbs to climb the stair. Scores: 1 = Rats unable to climb, revolved and oftentimes plopped. 2 = right side damaged when climbing or not clutching as tightly as the left side; 3 = rat climbing easily and clutching the stair tightly.

Body proprioception: each side of the body of rats was touched with a blunt stick and response to the stimulus was evaluated, scores: 1 = not responding to goad on the right side. 2 = slow response to stimulus on the right side, 3 = startled by the goad on both sides equally and turned its head.

Behavior of walk: spontaneous walking activity was checked. Scores: 0 = not walking, 1 = walks while wobbling; 2 = walks with shift to the right; 3 = walks rectilinearly ahead.

Head proprioception: sensory function was evaluated by brushing the vibrissae and checking the response on the left and right sides. Scores: 1 = no response to stimulus on the right side. 2 = Slow reaction to the stimulus on the right side; 3 = Turns its head to the stimulus side.

Finally, the scores of all these tests were summed, which resulted in a minimum score of 3 and a maximum score of 18 points overall.

2.5. Infarct Volume (TTC)

At the end of the ischemia/reperfusion (I/R) period (24 hours or 72 hours), rats were anesthetized by injection of chloral hydrate (350 mg/kg) and blood sampling was performed. Then, the rats were sacrificed, their brains were removed carefully and excised into 2-mm coronal sections. The brain slices were immersed in 2% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in normal saline solution at 37 ºC for 5 to 10 minutes. The mitochondrial enzyme in living cells reduced TTC to insoluble triphenylformazan crystals that stained normal brain tissue to red, but infarcted area and necrotic cells remained white. Photographs of the brain slices were evaluated by Optika Vision Pro software and infarct size was measured.

2.6. Real Time PCR

Gene expression of inflammatory factors, including Interleukin (IL)-1β, IL-6 and Tumor Necrotizing Factor-alpha (TNFα), were evaluated in the cortical cerebral tissue surrounding the ischemic lesion (penumbra) using real time polymerase chain reaction (PCR). The tissue samples were obtained from penumbra and frozen in liquid nitrogen quickly, and kept at -80°C until analysis. Total RNA was extracted from tissue by the RNA extraction kit (Peq gold; Germany). Then cDNA was synthesized from total RNA by AccuPower® RT-PCR PreMix (Bioneer; Korea). Real time PCR was performed by RealHelix™ qRT-PCR kit (Nano Helix; Korea).

Real time PCR reactions were performed in 8 × 6 (0.2 mL) strip (Applied Biosystem, USA) containing 5 μL of master mix (Nano Helix; Korea), 0.5 μL of both forward and reverse primer, 2 μL of diluted cDNA, and 2 μL water. Gene expression was measured using the Real time PCR technology (Applied Biosystem Step One Plus, USA) and a standardized protocol, as described previously (19). The primer sequences and annealing temperatures are given in Table 1.

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Table 1. Primer Sequences and Annealing Temperatures (AT) in Real-Time PCR

Gene		Primer Sequence	AT, °C	Size, bp
IL-1β	F	CTGTGACTCGTGGGATGATG	58	209
	R	GGGATTTTGTCGTTGCTTGT		
IL-6	F	CAAAAGAGAGCCTGGGACTG	58	209
	R	CAGAATTGCCATTGCACAAC		
TNFα	F	CTCCCAGAAAAGCAAGCAAC	60	210
	R	CGAGCAGGAATGAGAAGAGG		
HPRT	F	GCTGGTGAAAAGGACCTCT	60	284
	R	CACAGGACTAGAACACCTGC		

2.7. Enzyme Linked Immunosorbent Assay

Serum levels of inflammatory factors, interleukin (IL)-1β and Tumor Necrotizing Factor alpha (TNFα), were measured by Rat IL1β ELISA Kit and Rat TNFα ELISA Kit (Boster, USA) as described by the manufacturer's instructions. Blood samples were collected from heart of deeply anaesthetized rats at the end of reperfusion time and the serum was stored at -20°C before the analysis.

2.8. Statistical Analysis

Statistical differences between means of values were compared with One-Way Analysis Of Variance (ANOVA) test, followed by Tukey's post hoc test. Ordinal data of behavioral test were analyzed by the Kruskal-Wallis test. All data had a normal distribution. The normality of data was checked by the Kolmogrov-Smirnov test. All of statistical analyses were performed using SPSS software (version 22, Chicago, IL USA). Results with P value < 0.05 were considered as significant differences.

3. Results

3.1. Mesenchymal Stem Cells Characterization

Mesenchymal stem cells were characterized using three properties, including adherence to plastic, ability to differentiate to adipocytes and osteoblasts, and surface CD markers. Adherence to plastic was substantially used for separating MSCs. Bone marrow cells in the first passage are heterogeneous but after three passages, plastic adherent spindle-shape like cells spread in flasks (Figure 1A and B). Capability of differentiation to adipocyte and osteoblast cells was demonstrated by culturing of these cells in special differentiation media. Bone marrow-isolated cells were differentiated to osteoblast (Figure 1C) and adipocyte (Figure 1D) after 3 weeks. The purity of these MSCs was assessed by characterization of surface CD marker analysis

using flowcytometry, as previously described (20, 21). The presence of CD44 (98.3%), CD73 (75.4%), CD90 (99.5%), and CD105 (99.8%) and absence of hematopoietic markers, such as CD34 (4.52%) and CD45 (0.3%), in the surface of MSCs were demonstrated by this flowcytometry technique (Figure 1E).

3.2. Infarct Volume

The span of infarct lesions was indicated by staining of the brain section with 2,3,5-Triphenyltetrazolium Chloride (TTC). The size of the infarct area (white zone) and the total surface of the brain section were measured on slices, imaged by the Optika Vision Pro software and the ratio ($\times$ 100) was calculated and reported as percentage of infarct volume. Results indicated that infarct volume in the 24-hour MSC group (18.82 $\pm$ 1.58) was significantly lower than the 24-hour control (saline) group (27.4 $\pm$ 5.67; P < 0.01). Also, infarct volume in the 72-hour MSC group (14.4 $\pm$ 1.53) was significantly lower than in the 72-hour saline group (20.1 $\pm$ 2.77; P < 0.05) (Figure 2).

3.3. Behavioral Assessment

Garcia motor and sensory exams were performed in all groups, and total scores (max = 18) were compared. Results indicated that the total score of the Garcia behavioral test decreased in both the 24-hour (9.5 $\pm$ 2.3) and 72-hour (8.5 $\pm$ 3.2) saline groups in comparison to sham groups (17 $\pm$ 0.9; P < 0.001). Also, it demonstrated a significant increase in total scores of behavioral tests in the 72-hour MSC groups (14.5 $\pm$ 2.0; P < 0.01), yet not in the 24-hour MSC groups (13 $\pm$ 3.4) in comparison with the saline groups.

3.4. Enzyme Linked Immunosorbent Assay

Serum levels of IL-1β and TNFα were measured by ELISA method and the results indicated that serum level of IL-1β, 24 hours (1134 $\pm$ 241.3) and 72 hours (925 $\pm$ 196.8) after

Figure 1. Mesenchymal Stem Cells Were Isolated From Bone Marrow and Characterized by Morphology, Differentiation Ability and Surface CD Markers
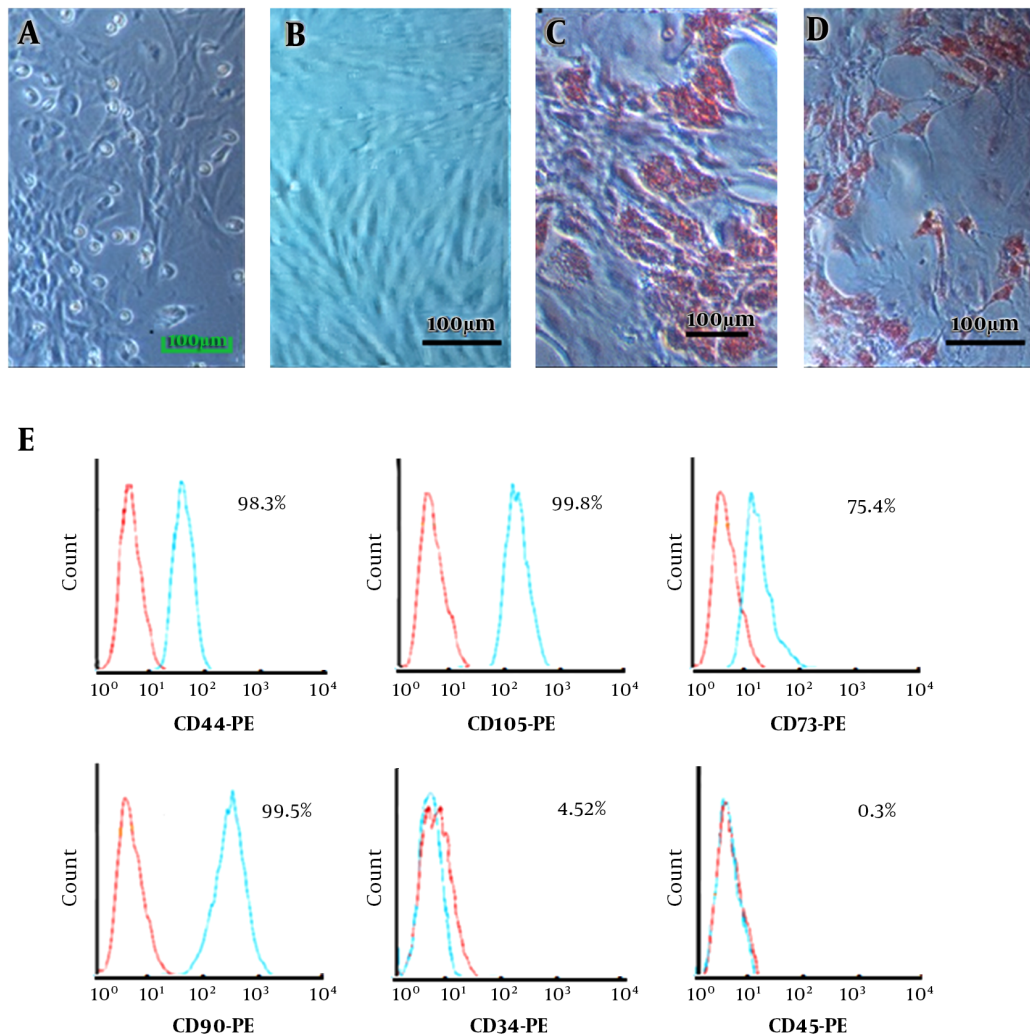
A, in first passage bone marrow derived cells are heterogeneous; B, after three passage MSCs (spindle form cells) were expanded. C, capability of differentiation to adipocyte; and D, osteoblast in specific media were checked. E, surface CD markers detected by flowcytometry; CD44, CD73, CD90, CD105 were present but CD34 and CD45 were absent.

I/R in the saline group was higher than the sham group after 24 hours (299.4 ± 79.3) and 72 hours (284 ± 107.5), respectively. However, serum levels of IL-1β in the MSC group were decreased in 24 hours (851.2 ± 134.3; P < 0.05) and 72 hours (368.3 ± 109.5; P < 0.001) in comparison to the saline group (Figure 3A). The assessment of TNFα serum levels in the 24-hour saline group (162.9 ± 31) and 72-hour saline group (202.6 ± 52) showed an increase towards the sham group (54.3 ± 11.3). However, TNFα serum levels in the 24-hour MSC group (133 ± 34.4) had not decreased significantly, while serum levels of TNFα in the 72-hour MSC group (126.9 ± 38.6) reduced in comparison with the saline

group, significantly (P < 0.01).

3.5. Real time Polymerase Chain Reaction

Inflammatory factor gene expression results were analyzed by the delta CT method (22), to determine the proportion of target gene delta CT to reference gene delta CT. The hypoxanthine-guanine phosphoribosyl transferase (HPRT) was used as a reference gene. Analysis of relative gene expression in each group was performed using the REST software (23). Results showed that the relative gene expression of IL-1β, IL-6, and TNFα which detected in both the 24-hour and 72-hour saline groups was significantly higher

Figure 2. The Garcia Motor and Sensory Tests Were Applied for Indicating Neurological Deficit
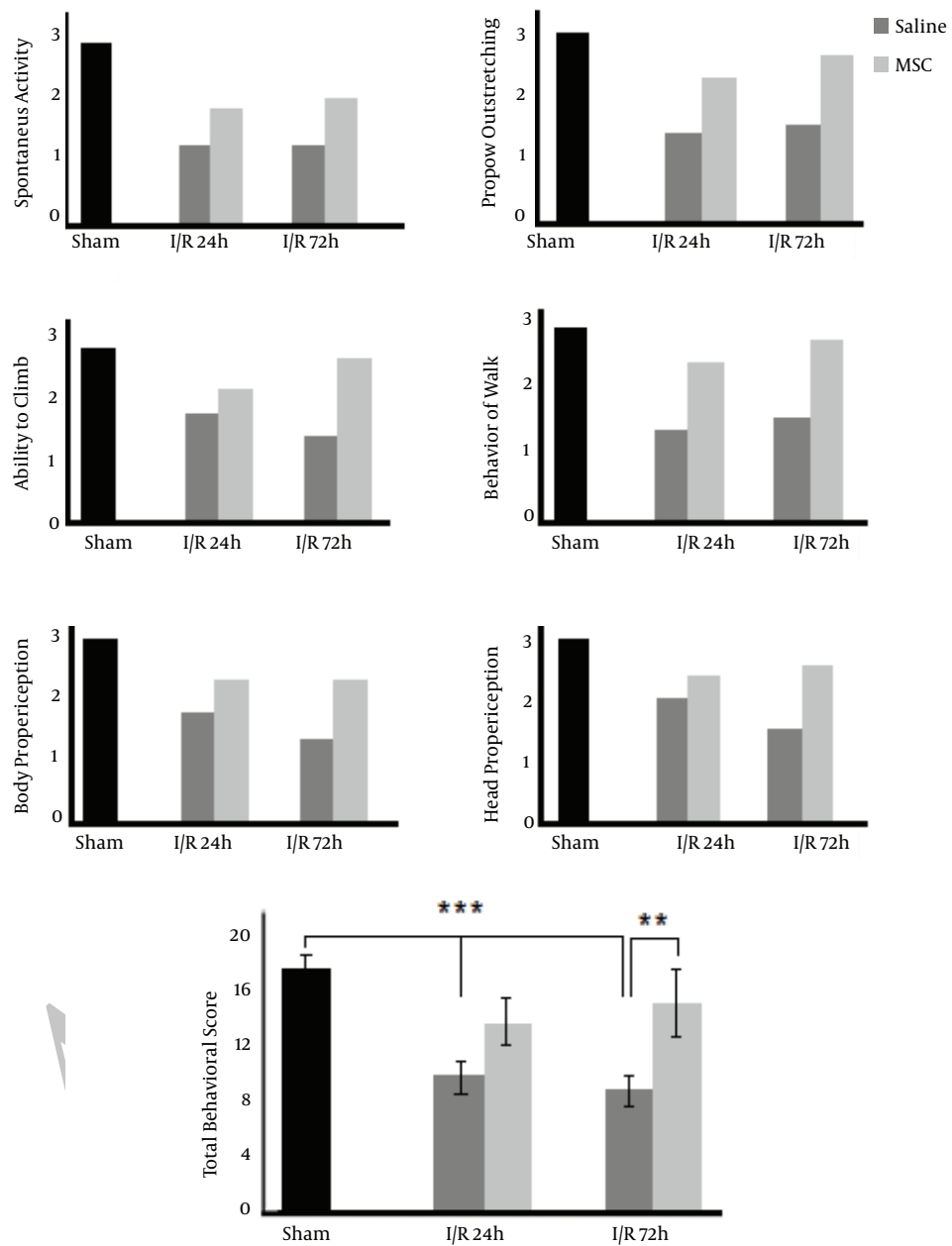
Scores of six parameters were compared and total scores (max = 18) were compared in all groups. Total scores in the saline groups 24 hours and 72 hours after tMCAO significantly decreased in the sham group (P < 0.001). No significant difference was found between saline and MSC groups 24 hours after reperfusion. However, after 72 hours, behavioral scores in the MSC group was increased significantly in comparison with the saline group (P < 0.01).

than sham group (P < 0.01). Moreover, relative gene expression of IL-1β in both the 24-hour (P < 0.01) and 72-hour (P < 0.05) MSC groups was significantly decreased in comparison to the 24-hour and 72-hour saline groups. Also, rel-
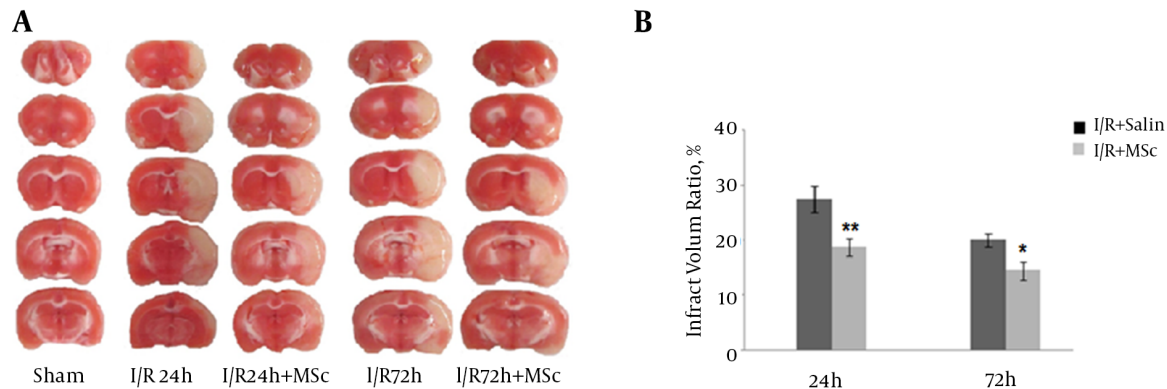
Infarct volume in the 24-hour MSC group was detected significantly higher than the saline group (P < 0.01) and in the 72-hour MSC group was measured higher than the saline group, significantly (P < 0.05).

ative gene expression of IL-6 in the MSC 24-hour and 72-hour groups was found to be significantly lower than in the saline group, respectively (P < 0.05). Again, the TNFα relative gene expression in the MSC 24-hour groups (but not 72-hour) was observed to be significantly lower than in the saline group (P < 0.05) (Figures 4 and 5).

4. Discussion

In the present study, neuroprotective effects of MSCs after ischemic stroke were investigated. The study focused on anti-inflammatory effects of MSCs and showed that inhibition of inflammation is a very important mechanism of neuroprotection by these cells. The results demonstrated that intravenous administration of bone marrow-derived MSCs could be preventing the expansion of infarct size and improve neurological defects after brain injury in the rat model of MCAO. This study confirmed recent experimental evidence indicating that MSCs transplantation has a therapeutic advantage in cerebral ischemia and other neurological disorders (24). Although mechanism of MSC neuroprotection is not fully understood, it is supposed that anti-inflammatory effects of MSCs have an important role in their neuroprotective properties.

The researchers quantified gene expression of pro-inflammatory factors in the penumbra region of infarct lesion, 24 and 72 hours after MCAO, demonstrating an immense increase in the gene expression of IL-1β, IL-6, and TNFα. Moreover, the experiment demonstrated that MSCs transplantation could decrease the expression of these pro-inflammatory factors in ischemic brain tissue. Immunomodulatory properties of MSCs have been demonstrated in previous studies yet the precise mechanism of

these effects remains unknown (25). These cells could release various molecules and proteins, which are involved in the inflammatory response. Most immune cells, including neutrophil, monocyte, macrophage dendritic cell, natural killer cell (NK), T and B lymphocyte, and regulatory T cell are impressed by MSCs (26, 27). Immunosuppression effects of MSCs could be achieved by cell-cell interaction or the release of several soluble factors, such as nitric oxide (NO), transform growth factor-β (TGF-β), Indole Amine 2,3-Dioxygenase (IDO) hepatocyte growth factor (HGF), prostaglandin E2 (28), HLA-G (29), CCL-2 (30), leukemia inhibitory factor (LIF) (31), IL1 receptor antagonist (IL1RA) (32), MMP-9 (33), hemeoxygenase-1, galectin-1, galectin-3, semaphorin-3A, and IL-10 (27, 34).

Several opinions are hypothesized for MSC-mediated neuroprotection, including release or activation of cytokines and growth factors, angiogenesis, differentiation of MSCs to neural or glial cells, and replacement of injured cells (35, 36). Some *in vitro* experiments demonstrated the immunosuppressive effects of BM-MSCs on subsiding lymphocytes alloreactivity in mixed lymphocyte reaction (MLR) assays (37). Nevertheless, a previous study revealed that the beneficial effects of MSC transplantation may not be through direct replacement of differentiated cells (38).

Studies have indicated that MSC therapy decreased the recruitment of microglia and macrophages to injured brain tissue and reduced infiltration of peripheral leukocytes to the damaged area (36). These properties maybe of help to inhibit ischemic lesion extension. Although invasion of immune cells helps in elimination of injured cell debris, excessive activity of immune cells exacerbated tissue damage. The current results demonstrated that MSCs

Figure 4. Serum Level of IL-1β Increased After 24 Hours and Remained High Until 72 Hours
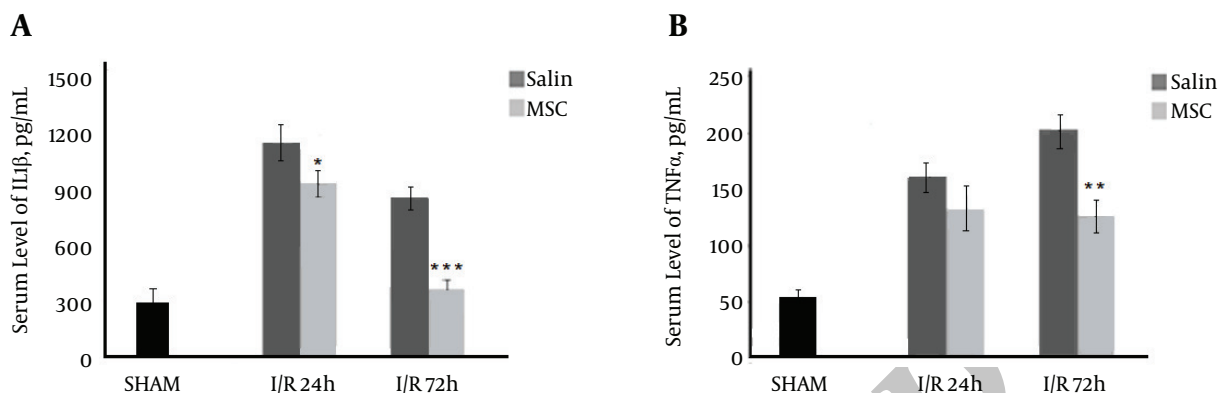
A, MSC transplantation decreased serum level of IL-1β in 24 hours (P < 0.05) and 72 hours (P < 0.001) after MCAO; B, Serum level of TNFα increased after 24 hours and 72 hours. MSC transplantation decreased the serum level of TNFα, which was significant only after 72 hours (P < 0.01).

Figure 5. Relative Gene Expression of IL-1β, IL- 6, and TNFα Increased After Ischemic Stroke (P < 0.01)
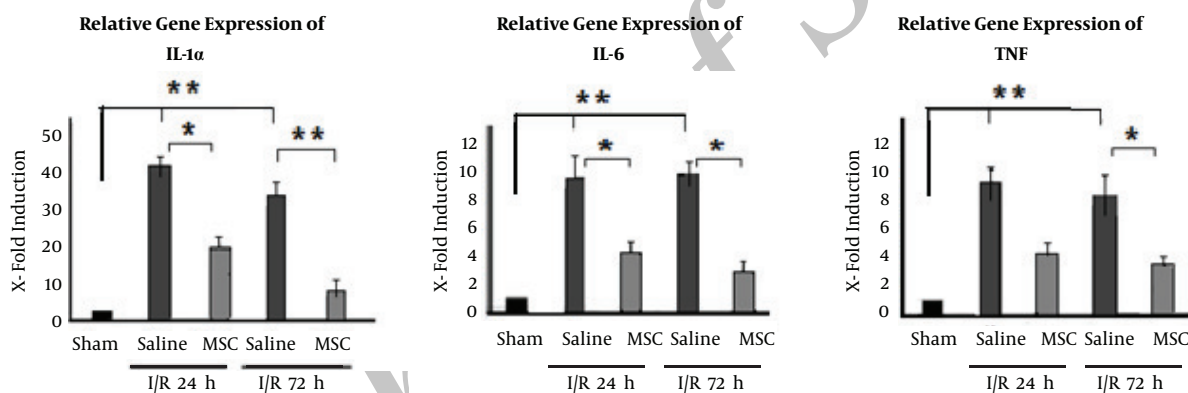
A, gene expression of IL-1β was decreased by MSC transplantation 24 hours (P < 0.05) and 72 hours (P < 0.01) after MCAO; B, Il-6 gene expression significantly decreased in the 24-hour and 72-hour MSC groups (P < 0.05); C, however, relative gene expression of TNFα in the MSC group 72 hours (but not 24 hours) after reperfusion was significantly lower than the saline group (P < 0.05).

transplantation decreased infarct volume at both 24 and 72 hours in the MSC group in comparison to the saline group. These results confirmed previous studies on the neuroprotective effects of MSCs in ischemic stroke.

Moreover, this study indicated that gene expression of pro-inflammatory cytokines, including IL-1β, IL- 6, and TNFα were increased in the penumbra of brain ischemic lesion. These cytokines were secreted by immune cells, such as macrophage, microglia, and T cells that were recruited in the brain after ischemic injury (39). Furthermore, serum levels of pro-inflammatory cytokines were measured for 24 and 72 hours after ischemia and results indicated an increasing systemic level of IL-1β and TNFα after ischemic stroke, and MSCs transplantation reduced the serum level of IL-1β after 24 and 72 hours, while serum level of TNFα

was decreased only 72 hours after MSCs transplantation. The current study focused on serum levels of cytokines because of similarity to patient sampling in the clinic. The researchers disregarded measurement of the IL-6 serum level, because this cytokine is secreted by MSCs as well as immune cells (40) and dissociating of its source is impossible. Moreover, it is suggested that measurement of serum levels of pro-inflammatory cytokines after stroke could be of help for recognition of patient status and treatment follow up beside other diagnostic methods, such as magnetic resonance imaging (MRI), computerized tomography (CT) scan, and behavioral motor sensory tests.

Also, a decrease in serum levels of pro-inflammatory cytokines after cell therapy is a good prognosis for post stroke recovery and displayed a successful treatment.

Some evidence indicated that MSCs promote the recovery of behavioral test and neurological defects after ischemic stroke (41, 42). The results of the present study indicated that motor and sensory neurological defects after MCAO in rats were improved by MSCs transplantation. Although neurological scores in MSC and saline groups 24 hours after MCAO did not display significant differences, yet rats in the MSC group, after 72 hours, had better results in the behavioral exam. It is probable that 24 hours is too soon for follow up of brain ischemic injury and therapeutic intervention, and delayed post reperfusion alterations caused secondary damage. It is suggested that anti-inflammatory properties of MSC have an important role in reducing brain injury and subsequent neurological defects.

The researchers administrated MSCs, 3 hours after reperfusion for investigation of MSCs effects in the early stage of acute ischemic stroke. However, several studies proved the beneficial therapeutic effects of these cells even several days after the prescription (2, 43).

4.1. Conclusions

In conclusion, anti-inflammatory properties of MSCs caused a decrease of brain injury and subsequent defects after MCAO in rat and affected improvement of ischemic stroke. Moreover, the increased serum levels of IL-β and TNFα after stroke were decreased by MSCs transplantation. Therefore, these cytokines could be used as markers for evaluating the effect of MSCs in improvement of ischemic stroke.

4.2. Limitations of the Study

This study investigated MCAO and stem cell therapy after 24 and 72 hours for acute and sub-acute response. The protective effects of MSc could be investigated in the chronic phase of stroke until 3 months after MCAO for late neuronal events, such as delayed neuronal death (DND).

Acknowledgments

Footnote

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