The effect of oral mucosal mesenchymal stem cells on long-term brain edema and lesion, anxiety-like behavior, and cognitive and motor outcomes in experimental traumatic brain injury

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Abstract

In recent years, the use of mesenchymal stem cells as a novel approach in the treatment of neurodegenerative diseases including traumatic brain injury has been proposed. In this study, the effect of oral mucosal mesenchymal stem cells **(**OMSCs) on traumatic brain injury was evaluated in long-term.

Animals were divided into 4 groups including sham, TBI, vehicle (Veh) and stem cell (SC). Brain damage was induced by the Marmarou's method. The number of 2×10^6 OMSCs was intravenously injected 1 and 24 hours after the injury. Brain edema and pathological outcome were assessed at 24 hours and 21 days after the injury. Besides, long-term neurological, motor and cognitive outcomes were evaluated at days 3, 7, 14, and 21 after the injury.

inflammation ($P < 0.01$), reduce axonal damage ($P < 0.01$, $P < 0.05$; respectively) and prevent microglia proliferation ($P < 0.05$, $P < 0.01$; respectively) at 24 h and 21 days after the injury. Neurological function improvement ($P < 0.001$), memory enhancement ($P < 0.05$), anxiety-like behavior reduction ($P < 0.001$) and motor function amelioration ($P < 0.05$, $P < 0.001$, $P < 0.01$, $P < 0.05$; respectively) at days of 3, 7, 14, 21 after the injury were observed in the treatment group.

According to the results of this study, OMSCs administration after TBI reduced brain edema and inflammation, and improved neurologic, memory and motor impairments, and anxiety-like behavior in long-term. Therefore, OMSCs could be a promising and new treatment option for TBI in the future.

Keywords: Traumatic brain injury; Stem cell, Brain edema; Inflammation; Neurological outcome; Cognitive outcome; Anxiety-like behavior

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1. Introduction

Traumatic brain injury is one of the major causes of death and disability worldwide [1, 2]. Despite the effective therapeutic options available, TBI treatment remains a challenge for scientists and physicians [3]. TBI commonly results in primary and secondary injury. Primary injury is caused by mechanical events, followed by a cascade of pathological and biochemical changes leading to secondary injury and neuronal death [4]. Increased reactive oxygen species and energy consumption [4], damaged blood-brain barrier [5], activated inflammation [6], progressive neuronal destruction [7], apoptosis and release of excitatory amino acids [8] are considered as mechanisms of secondary injury in TBI. These mechanisms lead to brain edema and increased intracranial pressure, which followed by neurological disorders [9] and cognitive impairments regardless of the severity of the injury [10]. Brain edema is a major cause of mortality and neurological disabilities caused by TBI [11].

Despite extensive investigations [12-14], sucssessful treatment for TBI has not yet been reported [3]. Stem cells (SCs) have currently been attracting a lot of interest in neuroscience research because of their important role in regenerative medicine [15]. Studies over the past decade have provided great support for the use of different stem cells in the treatment of neurological deficits such as TBI and stroke [16]. Among postnatal adult stem cells, mesenchymal stem cells (MSCs) with pluripotent property have of particular importance [8]. In addition to bone marrow, these cells have recently been isolated from other tissues including bone, primary teeth, placenta and oral mucosa [17] with properties similar to bone marrow

MSCs [18]. Also, they can differentiate into non-mesenchymal cells such as neural tissue [19]. These cells are in the focus of attention of regenerative medical studies due to their easy access, resource abundance, and high power of differentiation [16].

Among MSCs, oral mucosal mesenchymal stem cells (OMSCs) have been described as cells originating from the neural crest [17]. These cells are important for a wide range of scientific studies because of significant healing power, high SC population and availability due to being clonogenic cells [20], ability to differentiate into neural cells, fast proliferation, and stable morphology [17]. OMSCs can differentiate into astrocyte-like cells and are capable of producing neuroprotective factors such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), glia-derived neuroprotective factor (GDNF) and insulin-like growth factor-1 (IGF-1). Considering the neuroprotective effects of these cells in vitro [21] and in vivo [22], their study on the treatment of TBI has been proposed.

Administration of other types of stem cells including neural stem cells (NSCs) is also effective in treating TBI by enhancing cell proliferation, neurogenesis, and improving function [23]. In another study, bone marrow-derived MSCs decreased the permeability of the blood-brain barrier, neuronal inflammation and microglial accumulation in brain tissue [24]. Prompt administration of MSCs after injury intracranially enhances survival, proliferation, and differentiation of NSCs, and expression of NSCs-stimulating cytokines [25]. Consumption of human adipose-derived stem cells (hADSCs) improves cognitive deficits by reducing cortical lesion size and inhibiting cell death in the hippocampus [26].

Based on the absence of successful and effective treatment for TBI patients [8], the existence of evidence which shows that neuroprotective effects of MSCs on neurodegenerative

diseases including TBI [2], and also the benefits of using OMSCs in nervous diseases [22], the present study set out to assess the effect of OMSCs on TBI. In this investigation, the effect of OMSCs administration on brain edema, anxiety-like behavior, and pathological, neurological, cognitive, and motor outcomes induced by moderate diffuse TBI in male rats in long-term were evaluated.

2. Materials and Methods

2.1. Animals

This experimental interventional study was approved by the Ethical Committee of Kerman University of Medical Sciences with Code of Ethics IR.KMU.REC.1397.210. Animal care and behavioral tests were conducted in accordance with the standard ethical guidelines, and all efforts were made to minimize animal suffering. The experiments were performed on adult male Wistar rats weighing 200-220 g and bred in Kerman University of Medical Sciences. Animals were housed in a temperature-controlled room (25 $^{\circ}$C) for 12 h-light/dark cycle, with free access to water and food.

2.2. Experimental procedure

Animals were randomly divided into four groups: (*i*) Sham: rats received all necessary procedures to cause diffuse TBI except falling weight on their head, (*ii*)TBI: rats received a trauma by a 300 g weight on their head, (*iii*)Vehicle (Veh): rats intravenously received phosphate-buffered saline (PBS) in a volume of 100 µl at 1 and 24 hours after TBI [27], (*iv*) Stem cell (SC): rats intravenously received 2×10^6 OMSCs [28] in a volume of 100 µl at 1 and 24 hours after TBI.

2.3. Isolation of OMSCs

OMSCs were obtained from rat`s oral mucosa biopsies. The extracted tissue was washed with sterile PBS and enzymatically digested with 2% type I collagenase under shaking conditions. Collagenase activity was neutralized with an equal volume of low glucose Dulbecco's modified Eagle's medium (L-DMEM) containing 10% fetal bovine serum (FBS). To isolate OMSCs, the filtered cells were centrifuged at 2000 rpm/min for 10 min. The cell pellet was filtered by a 70 mm pore-size filter. The cells were cultured in L-DMEM supplemented PBS and 100 IU/mL penicillin + 100 µg/mL streptomycin during two weeks. The cells incubated at 36.5$^{\circ C}$, 5% CO2. The culture medium was changed every 72 h and the cells were passaged by trypsin enzyme [25]. In a pilot study, the number of 2×10^6 [28], 4×10^6 [29], and 8×10^6 OMSCs in 100 µl PBS was intravenously injected to rats (n=5 in each group) at 1 and 24 hours after TBI. Thereafter, their effects on brain edema, intracranial pressure (ICP), and histopathologic outcome were evaluated at 24 and 48 hours after TBI. Effective number of cells was determined when indices were compared to the control group.

2.4. Model of diffuse traumatic brain injury

Rats were anesthetized with the injection of ketamine (50 mg/kg) plus xylazine (10 mg/kg) intraperitoneally and then intubation of all animals was performed. The method was basically the same as described earlier [13]. A stainless steel plate 10 mm in diameter and 3 mm in thickness was attached to the skull bone between bergma and lambda. In all groups except the sham, a 300 g weight was dropped from a 2 m height onto the plate on the animal's head, causing diffuse TBI. Immediately thereafter, the rats were connected to a respiratory pump if it was needed. After restoration of spontaneous breathing, intratracheal tube was removed and the animals were placed in an individual cage following recovery of the surgery.

2.5. Evaluation of brain edema

The brain edema was evaluated by measuring brain water content at 24 h and 21day after TBI. Animals were anesthetized, the brains were removed, and brain samples were located in pre-weighed vials and weighed (wet weight). The lids were lifted and the vials were placed in an incubator at 100 $^{\circ}C$ for 48 h, and afterward reweighed (dry weight). The percentage of water in the brain of each animal was calculated as follows: (100 × [(wet weight–dry weight)/wet weight]) [30].

2.6. Evaluation of neurological severity score (NSS)

NSS was assessed on 3rd, 7th, 14th and 21st day after TBI by blind trained investigators to the experimental groups. NSS is an intricate behavioral test including motor, sensory, balance and reflex tests. Scoring range is 0–18, in which higher scores reflect a greater extent of the injury. The scores of 0 and 18 indicate normal performance and maximal impairment, respectively. The scores of 1–6, 7-12 and 13-18 indicate mild, moderate and severe injury, respectively (Table 1) [31].

Table 1. Neurological severity score

	Point
Motor tests	
Raising rat by the tail (normal = 0; maximum = 3)	
Flexion of forelimb	1
Flexion of hindlimb	1
Head moved >10° to vertical axis within 30 s	1
Placing rat on the floor (normal = 0; maximum = 3)	
Normal walk	0
Inability to walk straight	1
Circling toward the paretic side	2
Fall down to the paretic side	3
Sensory tests (normal = 0; maximum = 2)	
Placing test (visual and tactile test)	1
Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)	2
Beam balance tests (normal = 0; maximum = 6)	
Balances with steady posture	0
Grasps side of beam	1
Hugs the beam and one limb falls down from the beam	2
Hugs the beam and two limbs fall down from the beam, or spins on beam (>60 s)	3
Attempts to balance on the beam but falls off (>40 s)	4
Attempts to balance on the beam but falls off (>20 s)	5
Falls off: No attempt to balance or hang on to the beam (<20 s)	6
Reflexes absent and abnormal movements (normal = 0; maximum = 4)	
Pinna reflex (head shake when touching the auditory meatus)	1
Corneal reflex (eye blink when lightly touching the cornea with cotton)	1
Startle reflex (motor response to a brief noise from snapping a clipboard paper)	1

Seizures, myoclonus, myodystony	1
Maximum points	18

2.7. Behavioral assessment tests

In the present study, tests of elevated plus maze (EPM), open field (OFT) and Morris water maze (MWM) were done to assess anxiety-like behaviors, locomotor activity and spatial learning and memory, respectively. Behavioral tests were performed at days 3, 7, 14 and 21 after TBI. All sessions of behavioral tests were video-recorded by cameras which were hung from the ceiling (2.5 m high), and located directly above the center of the mazes. These cameras were connected to computers in a neighboring room for saving the rat's behavior. All behavioral indices were recorded by a video tracking system software (Borje Sanat, Iran). To avoid the effect of circadian rhythm on animal's behavior, the tests were performed at a determined time of day in a quiet environment.

2.8. Elevated plus maze (EPM)

An EPM apparatus was made of wood and consisted of two closed and two open arms with the equal size (50×10 cm). The close and open arms were enclosed by 40-cm-high and 0.5-cm-high walls, respectively. The four arms were linked by a central platform (10×10 cm). The apparatus was elevated 50 cm above the floor. The experiments were performed in a room lit by a 60-W light bulb located above the center of the EPM. The animals were placed in a room for acclimation 1 h before behavioral testing without observing the apparatus. Each rat was placed in the center of the EPM facing an opened arm and allowed 5 min of exploration. Entry was defined as four paws in the arms. The number of entries into close and open arms and the total time spent in the closed and open arms were measured. As the anxiety indices, the percentage of open arm

time (%OAT: the ratio of times spent in the open arms to total times spent in any arms×100) and open arm entries (%OAE: the ratio of entries into open arms to total entries×100) were calculated. Moreover, total arm entries were evaluated as a relative pure parameter of locomotor activity [32].

2.9. Open field test (OFT)

An OFT apparatus was constructed of plexiglass and consisted of a square arena (90 × 90 × 30 cm), which was divided by lines into 16 equal squares. Each rat was located in the central zone and allowed 5 min of exploration. All experiments were performed in a dimly illuminated testing room. The velocity (cm/s) of animals and total distance moved (cm) were measured [33].

2.10. Morris water maze (MWM)

The Morris water maze is an authentic apparatus to assess spatial learning and memory in laboratory animals. This task is a circular tank, 150 cm in diameter and 60 cm in depth, filled with water (23- 25°C). The animals ran away from water onto an invisible platform (10 cm wide, 35 cm high), which located 1.5 cm beneath the water level. The MWM was surrounded by different visual cues on the testing room walls, and their place remained unchanged throughout the test period. The maze was divided into four quadrants, and the animals were placed in one of the four equal quadrants, randomly. The parameters such as the total time spent in the target quadrant and the number of entries to the target quadrant were measured. The training session included three blocks on three consecutive days and each block comprised of four consecutive trials. On each trial, rats were randomly dropped into the maze from a defined point of each quadrant and were allowed to swim for 60 seconds to find the hidden platform. After the detection of the platform, the animal remained there for 20–30 s and then was caged for 20–30 s

before the next trial. The retention of spatial memory was assessed 24 h after training trials by removing the platform in a 60 s probe trial [34].

2.11. Histopathology

The histopathological outcome was evaluated on the first and 21st day after TBI. Briefly, the brain tissue was washed with 0.9% cold saline, fixed in 10% formalin and, after tissue processing, embedded in paraffin and coronally sectioned into 5μm. Slides were prepared and stained with hematoxylin and eosin (H&E). Axonal degeneration, inflammation and microglia proliferation in the brain tissue were microscopically assessed by a pathologist who was blind to the experimental groups. The histopathologic changes in the brain tissue were examined semi-quantitatively and graded as follows: (0) nil = unchanged brain tissue, (1) mild = 1-29% changes in the brain tissue, (2) moderate = 30-59% changes in the brain tissue, (3) severe = 60-100% changes in the brain tissue [35].

2.12. Statistical analysis

Results were reported as mean±SEM. Normality of data was checked using the Shapiro-Wilk test. Data with normal distribution was analyzed using Two way repeated measures ANOVA to compare mean data between groups at different times, except for variables of brain water content and histopathologic indices. The analysis of latter indices was performed using one-way ANOVA. In all statistical comparisons, p values less than 0.05 were considered as the criterion for statistical significance. Data analyses were done using the SPSS software package version 20 (SPSS Inc, Chicago, IL, USA).

3. Results

3. 1. OMSC administration reduced the brain water content after TBI

The comparison of brain water content between the experimental groups was performed at 24 hours and 21 days after trauma. As shown in Fig. 1, brain water content increased after brain injury compared to the sham group at both times ($P < 0.001$). The administration of SC lessened the brain water content compared to the vehicle group at 24 hours and 21 days after injury ($P < 0.001$). At 24 hours and 21 days after injury, the brain water content in the group receiving SC did not differ from the sham group.

3.2. OMSC administration ameliorated long-term neurological outcome

The analysis showed that there was an interaction between group and time for NSS variable ($P < 0.001$), and the main effect of score for both time and group was $P < 0.001$. At all times after injury, a significant increase in NSS was observed in the TBI and vehicle groups compared with the sham group ($P < 0.001$). NSS was higher in SC group compared to the sham group ($P < 0.001$). On the other hand, SC group was able to significantly reduce the amount of NSS compared to the vehicle group ($P < 0.001$) (Fig. 2).

3.3. OMSC improved anxiety-like behaviors in the EPM after TBI

In this study, %OAT, %OAE and locomotor activity as indices of anxiety-like behaviors in the EPM were assessed on days 3, 7, 14 and 21 after injury. The analysis showed that there was an interaction between group and time for %OAT variable ($P < 0.001$). The main effect of %OAT for both time and group was $P < 0.001$. Fig. 3A illustrates the percentage of time spent in the open arm of the EPM in study groups. %OAT decreased in the TBI and vehicle groups compared with the sham group at all days after injury ($P < 0.001$). The %OAT was significantly increased

in the SC group compared to the vehicle group at days 3 ($P < 0.01$), 7, 14, and 21 after injury ($P < 0.001$).

The analysis showed that there was no interaction between group and time for %OAE ($P=0.52$). Main effect of %OAE for time and group was $P=0.68$ and $P<0.001$, respectively. A comparison of the %OAE in study groups is shown in Fig. 3B. This variable was lower in the TBI and vehicle groups than in the sham group ($P < 0.001$). The %OAE increased in the SC group compared to the vehicle group ($P < 0.001$).

There was no interaction between group and time for locomotor activity ($P = 0.57$), and main effect of motor activity for time and group was $P < 0.001$ and $P = 0.22$, respectively. A comparison of the locomotor activity in sham, TBI, vehicle and SC groups is shown in Fig. 3C. No significant difference was seen among groups.

3.4. OMSC administration reversed the TBI-induced velocity decline in the OFT

The velocity in the OFT was evaluated as the index of motor outcome. The analysis showed that there was an interaction between group and time for velocity variable in the OFT ($P < 0.001$) and the main effect of velocity for both time and group was $P < 0.001$. Fig. 4A shows the comparison of velocity in study groups at 3, 7, 14 and 21 days after injury. Velocity decreased in TBI and vehicle groups compared with sham group at days of 3 ($P < 0.01$), 7 ($P < 0.01$, $P < 0.001$), 14 ($P < 0.01$, $P < 0.001$) and 21 ($P < 0.01$, $P < 0.05$), respectively. The velocity increased in the SC group compared to the vehicle group at 3 ($P < 0.05$), 7 ($P < 0.001$), 14 ($P < 0.01$) and 21 ($P < 0.05$) day after injury.

The analysis showed that there was no interaction between group and time for traveled distance ($P = 0.53$), and main effect of traveled distance for time and group was $P < 0.01$ and P

=0.42, respectively. A comparison of the traveled distance in sham, TBI, vehicle and SC groups is shown in Fig. 4B. No significant difference was seen between groups.

3.5. OMSC partly improved spatial memory in the MWM after TBI

Spatial memory was assessed using the time spent in the target quadrant and the number of entries to the target quadrant during probe trial in the MWM (Fig. 5). No significant difference for distance traveled in the target quadrant was found (Data not shown).

The analysis showed that there was no interaction between group and time for the target quadrant during probe trial (P=0.94). Main effect of time spent in the target quadrant for time and group was P=0.85 and P=0.002, respectively. The time spent in the target quadrant during probe trial in study groups is illustrated in Fig. 5A. This time decreased in the TBI and vehicle groups compared to the sham group (P <0.01). This parameter index increased in the SC group compared to the vehicle group (P <0.01).

The analysis showed that there was no interaction between group and time for the number of entries to the target quadrant (P=0.15). The main effect of the number of entries to the target quadrant for the time and group was P=0.34 and P=0.002, respectively. Fig. 5B shows the number of entries to the target quadrant during probe trial in study groups. This variable decreased in TBI and vehicle groups compared to the sham group (P <0.05). This parameter increased in the SC group compared to the vehicle group (P <0.05).

3.6. Histopathological results

To evaluate the pathologic outcome, parameters including axonal degeneration, the degree of inflammation, and microglia proliferation in the brain tissue were evaluated (Fig. 6).

Histopathological images (haematoxylin & eosin, 400×) in sham, vehicle, traumatic brain injury, and stem cell groups are shown in Fig. 6A.

At 24 h and 21 days after injury, axonal degeneration increased in the TBI and vehicle groups compared with the sham group ($P < 0.01$) (Fig. 6B). Although axonal degeneration was in the SC group higher than that the sham group at 24 hours and 21 days after injury ($P < 0.01$), this variable was low in the SC group compared to the vehicle group at 24 hours ($P < 0.01$) and 21 days ($P < 0.05$) after injury.

At 24 hours and 21 days after injury, inflammation score increased in the TBI and vehicle groups compared with the sham group ($P < 0.05$) (Fig. 6C). The score of inflammation in the SC group decreased compared to the vehicle group at 24 hours and 21 days after injury ($P < 0.01$).

Fig. 6D illustrates the results of microglia proliferation score in study groups at 24 hours and 21 days after injury. At 24 hours after injury, the score of microglia proliferation significantly increased in the TBI and vehicle groups compared with the sham group ($P < 0.05$). This increment was also observed at 21 days after injury in the TBI ($P < 0.05$) and vehicle ($P < 0.01$) groups compared with the sham group. As shown in Fig. 6D, microglia proliferation in the SC group decreased compared with the vehicle group at 24 hours ($P < 0.05$) and 21 days ($P < 0.01$) after injury.

4. Discussion

In the present study, the administration of OMSCs could improve neurological, cognitive and motor functions, and anxiety-like behavior in TBI probably by decreasing cerebral water content. Therefore, the administration of OMSCs was successful in improving long-term outcomes of TBI. Given the high mortality rate and life-long disabilities, lack of effective and definitive treatments [36], and suggestion of stem cell application due to heterogeneous nature of

TBI [8], the effect of OMSCs on cerebral edema, anxiety-like behavior and long-term neurological, pathological, cognitive, and motor outcomes of diffuse TBI were investigated for the first time in the present research. In the present study, an intravenous injection of OMSCs was used. Studies on using SC for TBI suggest that the best way of carrying SC to the injury site is IV injection, though the studies have shown that only 1% of the injected cells can reach the injury site in the brain [37-40].

Further studies are needed to find the appropriate time for SC injection [41]. A study showed that injection of SC after 24 hours of cerebral injury decreased the neuronal inflammation and increased the angiogenesis and neurogenesis [38]. In another study, exosome injection of MSCs after 15 minutes from the TBI could inhibit the inflammation by disturbing the injury cycle [41]. On the other hand, other studies stated that early administration of SC could not be effective due to the tissue inflammation, and delayed administration or repeating the dose may result in more effective outcomes [2, 42]. This study investigated repeating the injection of OMSCs in 1 and 24 hours after the injury.

In the present study, the increase in cerebral water content was prevented using OMSC. In agreement with our research, many studies have shown the increased cerebral water content after TBI [43, 44]. One of the main causes of developing cerebral edema is the increased inflammatory responses and the activation of inflammatory cascades that result in disruption of the BBB and further introduction of inflammatory agents and immune cells and consequently the development of cerebral edema [9]. In a study, it was reported to be increased expression of tissue inhibitor of metalloproteinase 3 (TIMP3) by MSC, leading to a decrease in the permeability of the BBB in murine TBI model and thus improving the recovery after injury [45]. The reduction of brain inflammation was appeared in SC group in this study like brain edema

results. Therefore the administration of OMSCs after TBI probably inhibited the development of brain edema partly by decreasing brain inflammation.

Function improvement following the administration of MSCs is thought to be caused by decreased inflammatory and oxidant factors, and also neurogenesis stimulation [41]. Motor dysfunctions are TBI common complications [46]. In the present study, the administration of OMSCs improved the motor impairments in the animals of the treatment group so that the reduction in motor speed was resolved. Motor function improvement of the treatment group was also reported in the experimental study using NSCs for treating post-TBI cognitive and motor deficits [47]. Another study reported that embryonic stem cell administration after cerebral injury improved the motor function in mice [48]. The effectiveness mechanism of MSCs on post-traumatic motor outcomes is not completely understood. MSCs may improve short-term motor function by inducing neurogenesis and axonal repair, improving tissue condition, and thus providing a better environment to advance tissue regeneration. It is albeit well demonstrated that administrating MSCs can significantly decrease the primary post-traumatic inflammation [41] so that the animal motor function is improved; however, the molecular mechanism is unknown.

One of the important and common complications of TBI is also anxiety, which constitutes a significant part of the long-term symptoms and complications of TBI [49]. In the present study, post-traumatic anxiety-like behavior was indicated by a decrease in time spent in the open arms and a reduction in the entries into the open arms of EPM. Administration of OMSCs increased the time spent in and the number of entries the open arms, indicating a reduction in anxiety in the treatment group. Based on the studies and clinical trials done, TBI-induced anxiety and other psychological disturbances seem to be associated with the injury to the posterolateral area of the

prefrontal lobe and the left side of basal nuclei. Progressive atrophy induced by injury in the area results in decreasing the recovery speed and defecting the cerebral function [50].

In line of our results of memory, administration of mouse neural stem / progenitor cells (NSPCs) to mice with TBI could improve memory [51], whereas human NSPC, regardless of its positive efficacy and relieving motor deficits, had no effect on the development of long-term cognitive activity and memory [52]. In a study conducted by Zhou et al. (2019), it was shown that MSCs administration ameliorated neurological dysfunction and also memory and learning impairment after TBI [53]. Transplantation of MSCs overexpressing fibroblast growth factor 21 (FGF-21) as a neuroprotective protein recovered hippocampal-dependent and independent learning and memory deficits in a mouse model of TBI [54]. In a study, it was shown that BMSCs transplantation could improve cognitive impairment through up-regulation of hippocampal GABAergic system in a rat model of chronic cerebral hypoperfusion [55].

In the present study, the neuronal damage and microglia proliferation was inhibited by the stem cell administration. Several studies demonstrated that MSCs could reduce inflammation following cerebral injury [29, 41, 42]. In a recent study, the administration of MSCs was found to significantly increase the *in vivo* and *in vitro* expression of IL-10 in the injured tissue. Treatment with MSCs also improved the motor skills and reduced the number of activated astrocytes and macrophages, thereby preventing the tissue from dying. MSCs can contribute to the regulation of dendritic cells, macrophages, and natural killer cells secreting cytokines [56]. They directly inhibit the proliferation of T cells and microglial cells and also decrease the secretion of inflammatory cytokines by dendritic cells, monocytes, and macrophages [57]. Moreover, MSCs can immigrate to the injured tissue [42]. They inhibit the pro-inflammatory cytokine activities and proliferation of T lymphocytes and microglial cells in the tissue. Also,

MSCs increase the survival rate of the damaged cells by releasing the anti-inflammatory cytokines and immunologic regulatory factors [57]. The associated mechanism of action of stem cells is not fully known. It has been stated that after immigration to the injured tissues, these cells increase the immunologic tolerance in the environment and also the survival rate of the injured cells by inhibiting the release of pro-inflammatory cytokines [24].

5. conclusion

The cerebral edema caused by TBI was reduced following SC administration partly by decrasing inflammation. Also, SC administration prevented long-term motor, cognitive and neurologic dysfunction and anxiety following TBI probably due to inhibiton of brain edema and inflammation. Given the study results, we suggest that the use of OMSCs should be noted for prevention from long-term impairments of TBI and maybe other neurodegenerative disorders in future studies. Since the mechanism of action of MSC is not fully understood, given the abundant number of unanswered questions in this field, further studies are needed to understand the involved mechanisms and prove the safety of the method.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Funding

Not applicable

Ethics Approval

The study was approved by the Ethical Committee of Kerman University of Medical Sciences with Code of Ethics IR.KMU.REC.1397.210.

Consent for publication

Not applicable

Acknowledgments

This research article is a part of thesis product of Mrs. Fatemeh Dehghanian. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Fatemeh Dehghanian: Collection of data

Zahra Soltani: Conception and design, Data analysis and interpretation, Manuscript writing, Final approval of manuscript

Alireza Farsinejad: Conception and design

Elham Jafari: Collection of data

Hamideh Bashiri: Manuscript writing

Data Availability Statement

Not Applicable

Abbreviations

BDNF: brain-derived neurotrophic factor; EPM: elevated plus maze; FBS: fetal bovine serum; FGF-21: fibroblast growth factor 2; GDNF: glia-derived neuroprotective factor; hADSCs: human adipose-derived stem cells; H&E hematoxylin and eosin; ICP: intracranial pressure; IGF-1: insulin-like growth factor-1; L-DMEM: low glucose Dulbecco's modified Eagle's medium; MWM: Morris water maze; MSCs: mesenchymal stem cells; NSPCs: neural stem / progenitor cells; NSS: neurological severity score; NSCs: neural stem cells; OAE: open arm entries; OAT: open arm time; OFT: open field; OMSCs: oral mucosal mesenchymal stem cells; PBS: phosphate-buffered saline; SCs: Stem cells; TBI: Traumatic brain injury; TIMP3: tissue inhibitor of metalloproteinase 3; VEGF: vascular endothelial growth factor; Veh: Vehicle;

Legends

Fig. 1. Comparison of brain water content (%) in study groups at 24 h and 21 day after injury (n = 7). Each bar represents mean ± SEM. ***p < 0.001 compared with the sham group. ^^^p < 0.001 compared with the vehicle group. TBI: traumatic brain injury; Veh: vehicle; SC: stem cell

Fig. 2. Comparison of neurological severity score (NSS) in study groups at 3, 7, 14 and 21 days after injury (n = 7). Each bar represents mean ± SEM. ***p < 0.001 compared with the sham group. ^^^p < 0.001 compared with the vehicle group. TBI: traumatic brain injury; Veh: vehicle; SC: stem cell

Fig. 3. Comparison of open arm time (%) (3A), open arm entry (%) (3B) and locomotor activity (3C) in the elevated plus maze (EPM) in study groups at 3, 7, 14 and 21 days after injury (n = 7). Each bar represents mean ± SEM. *p < 0.05, ***p < 0.001 compared with sham group. ^^p < 0.01, ^^^p < 0.001 compared with vehicle group. TBI: traumatic brain injury; Veh: vehicle; SC: stem cell

Fig. 4. Comparison of velocity in the open field test (OFT) (4A) and traveled distance (4B) in study groups at 3, 7, 14 and 21 days after injury (n = 7). Each bar represents mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 compared with sham group. ^p < 0.05, ^ ^p < 0.01, ^^^p < 0.001 compared with vehicle group. TBI: traumatic brain injury; Veh: vehicle; SC: stem cell

Fig. 5. Comparison of time spent in the target quadrant during probe trial in Morris water maze (MWM) (5A) and the number of entries to target quadrant during probe trial (5B) in study groups at 3, 7, 14 and 21 days after injury (n = 7). Each bar represents mean ± SEM. *p < 0.05, **p < 0.01 compared with sham group. ^p < 0.05 compared with vehicle group. TBI: traumatic brain injury; Veh: vehicle; SC: stem cell

Fig. 6. Histopathological images (haematoxylin & eosin, 400×) in study groups, microglia (thick arrow), axonal degeneration (medium arrows), inflammation (thin arrows) (6A). Comparison of axonal degeneration (6B), inflammation score (6C) and microglia proliferation score (6D) in study groups at 24 h and 21 days after injury (n = 7). Each bar represents mean ± SEM. *p < 0.05, **p < 0.01 compared with the sham group. ^p < 0.05, ^^p < 0.01 compared with vehicle group. TBI: traumatic brain injury; Veh: vehicle; SC: stem cell

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Fig. 1

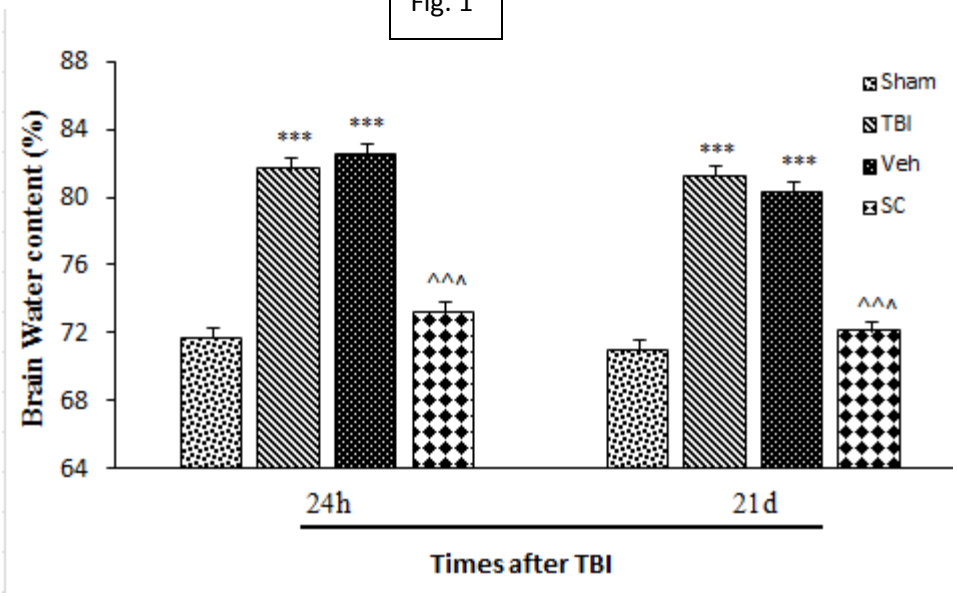

Brain Water content (%)
88
84
80
76
72
68
64
***
***
***
***
^^^
^^^
Sham
TBI
Veh
SC
24h
21d
Times after TBI

Fig. 2

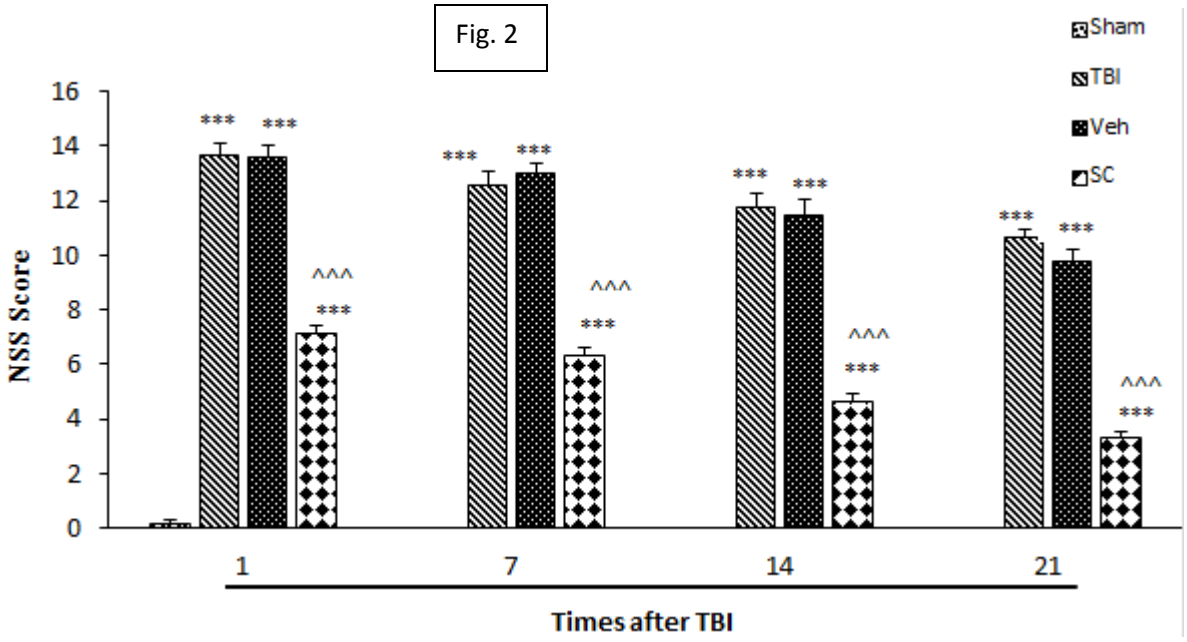
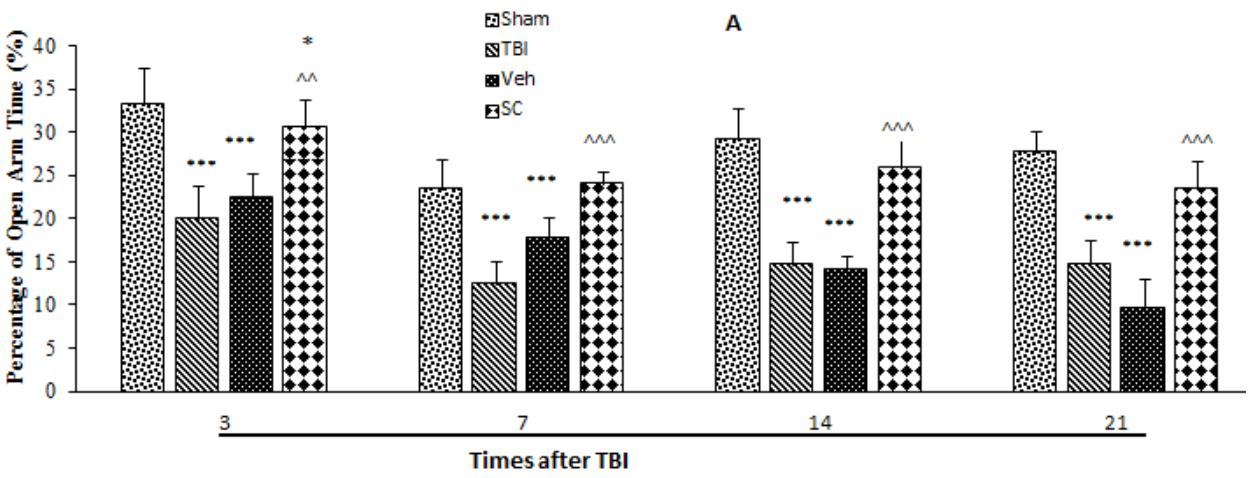
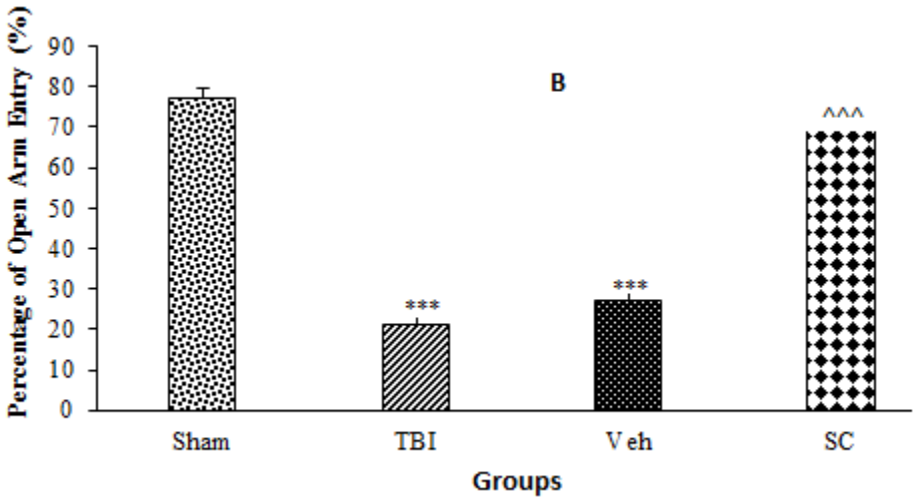

NSS Score
16
14
12
10
8
6
4
2
0
***
^^^
Sham
TBI
Veh
SC
1
7
14
21
Times after TBI

Fig. 3


A
Sham
TBI
Veh
SC
Percentage of Open Arm Time (%)
0
5
10
15
20
25
30
35
40
*
^^
***
^^^
3
7
14
21
Times after TBI


B
Percentage of Open Arm Entry (%)
0
10
20
30
40
50
60
70
80
90
***
***
^^^
Sham
TBI
Veh
SC
Groups

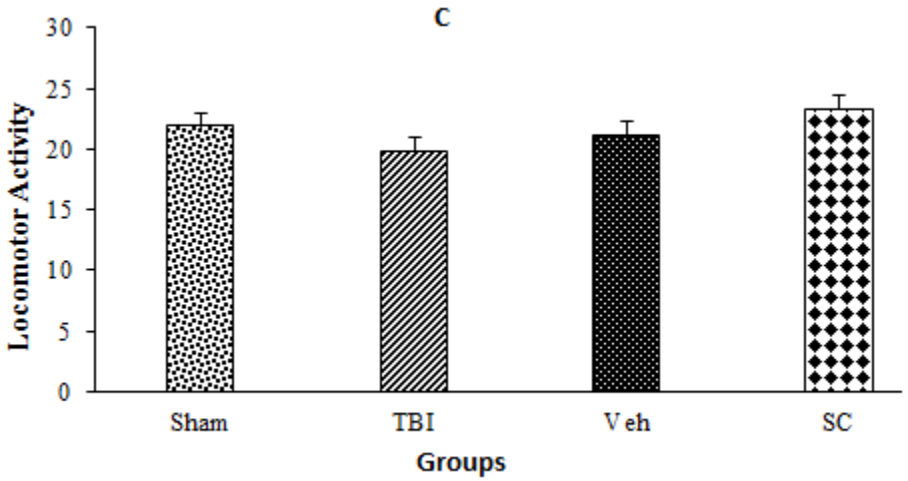

C
Locomotor Activity
0
5
10
15
20
25
30
Sham
TBI
Veh
SC
Groups

Fig. 4

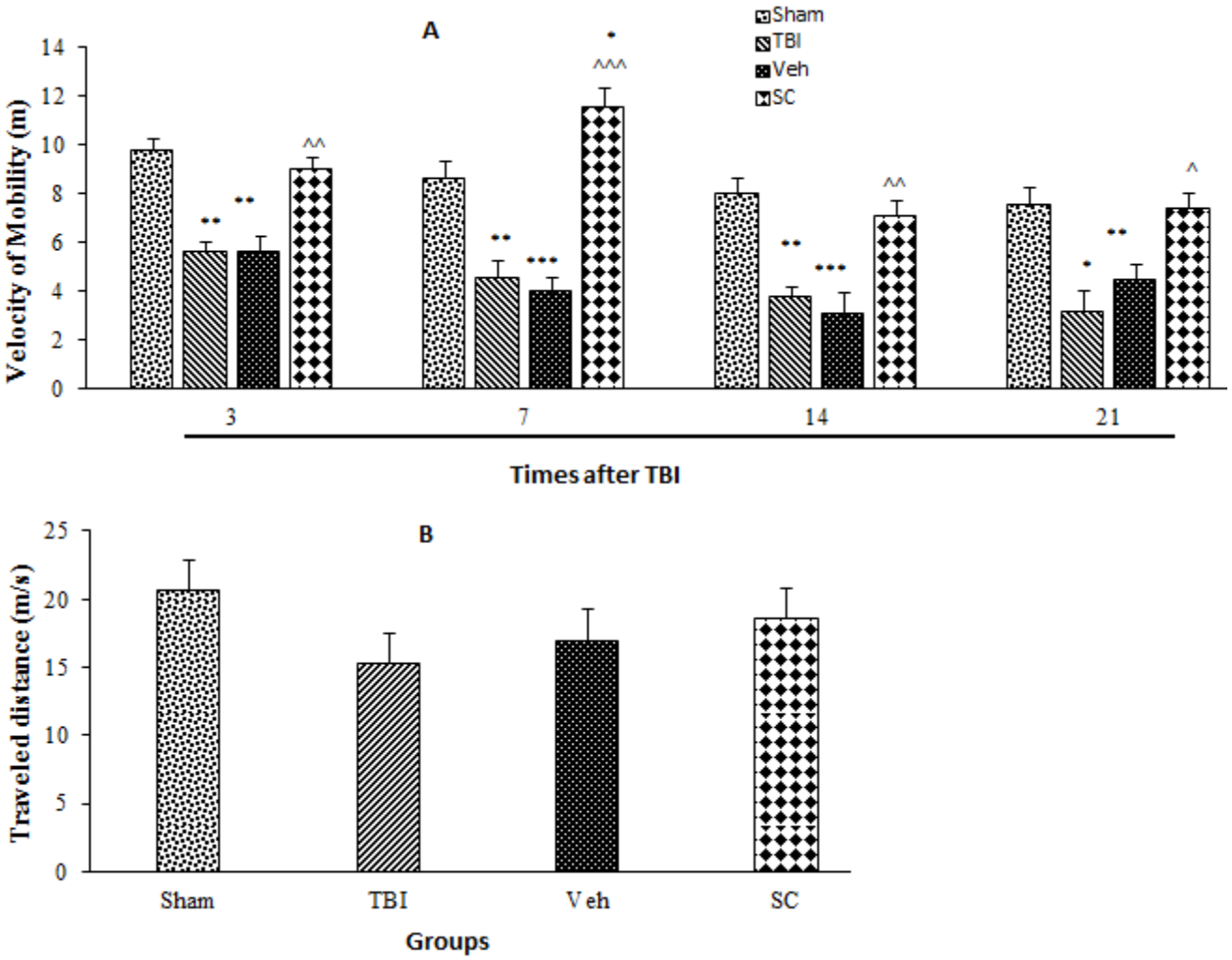

A
Sham
TBI
Veh
SC
Velocity of Mobility (m)
14
12
10
8
6
4
2
0
3
7
14
21
Times after TBI
B
Traveled distance (m/s)
25
20
15
10
5
0
Sham
TBI
Veh
SC
Groups

Fig. 5

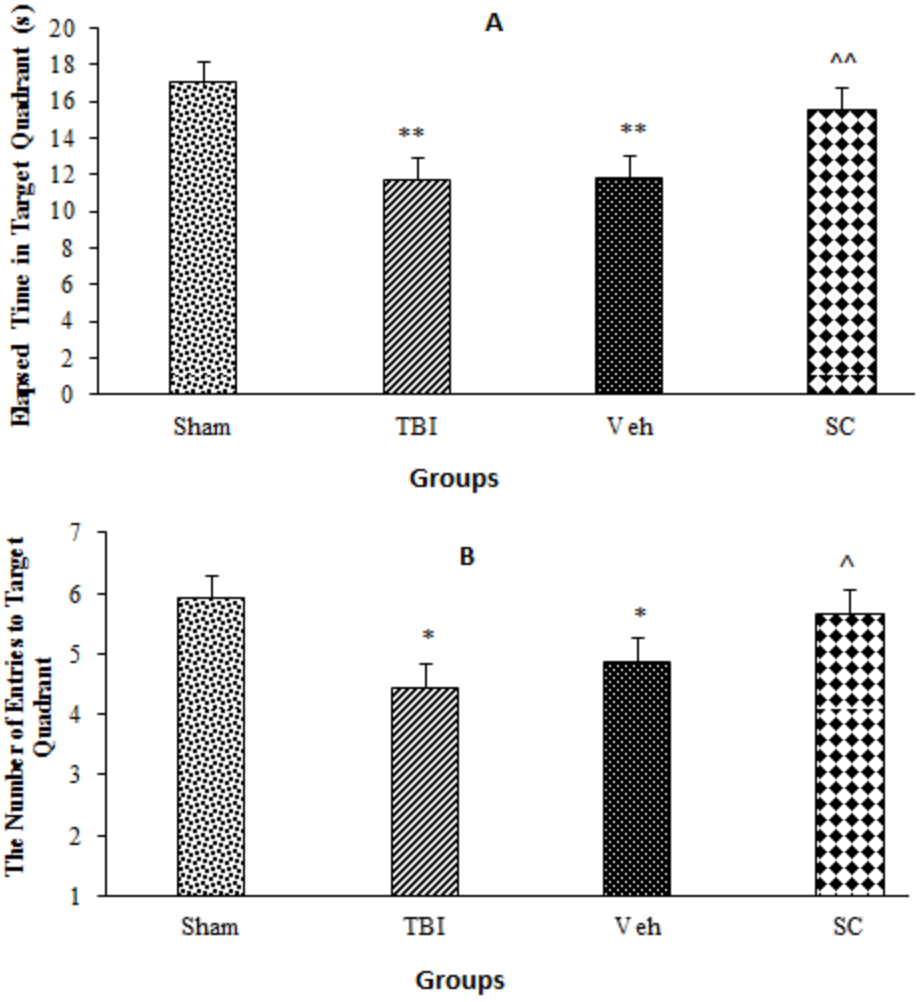
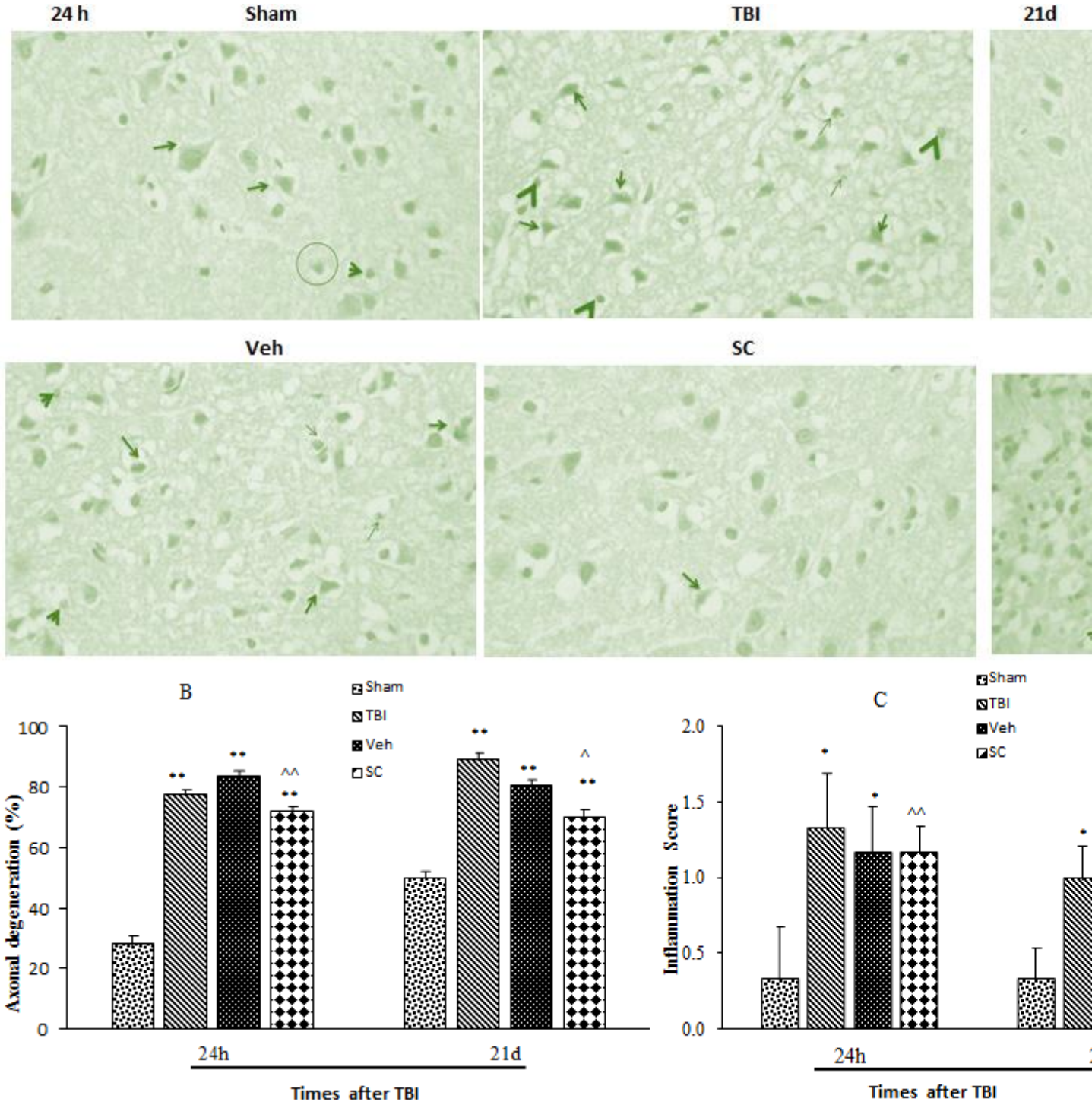

A
Elapsed Time in Target Quadrant (s)
20
18
16
14
12
10
8
6
4
2
0
**
**
^^
Sham
TBI
Veh
SC
Groups
B
The Number of Entries to Target Quadrant
7
6
5
4
3
2
1
*
*
^
Sham
TBI
Veh
SC
Groups

Fig. 6


24 h
Sham
TBI
21d
Veh
SC
B
Sham
TBI
Veh
SC
Axonal degeneration (%)
100
80
60
40
20
0
24h
21d
Times after TBI
C
Sham
TBI
Veh
SC
Inflammation Score
2.0
1.5
1.0
0.5
0.0
24h
Times after TBI