Original Article

Intravenous Injection of Human Umbilical Cord Matrix Stem Cell (Wharton Jelly Stem Cell) Provides Functional Recovery in a Rat Model of Traumatic Brain Injury

Mohsen Marzban, M.Sc.[1*], Mehrdad Bakhtiary, Ph.D.[1, 2], Mehdi Mehdizadeh, Ph.D.[1, 2], Mohamad Taghi Joghataei, Ph.D.[1, 2], Samideh Khoei, Ph.D.[3], Vahid Pirhajati Mahabadi, M.Sc.[1], Seyed Jafar Hashemian, M.D.[1], Mahdi Tondar, B.Sc.[1], Bahareh Laribi, M.Sc.[4], Asghar Ebrahimi, M.Sc.[5], Babak Abhari, M.D.[1]

1. Anatomy Department, Iran University of Medical Science, Tehran, Iran
2. Cellular and Molecular Research Center, Iran University of Medical Science, Tehran, Iran
3. Medical Physics Department, Iran University of Medical, Tehran, Iran
4. Immunology Department, Iran University of Medical Science, Tehran, Iran
5. Biotechnology and Bioscience Department, Milano-Bicocca University, Milan, Italy

**** Corresponding Address: P.O.Box: 14155-6183, Anatomy Department, Iran University of Medical Science, Tehran, Iran***
Email: mohsen136051@yahoo.com

Received: 5/Jan/2009, Accepted: 24/Jun/2009

Abstract

Objective: This study was designed to examine the effects of human umbilical cord matrix stem cell (hUCMSC) administration in rats for 6 weeks after traumatic brain injury (TBI).
Materials and Methods: Adult male Wistar rats (n = 30) were injured with controlled cortical impact device and divided into three groups. The treatment group (n = 10) was injected with 2 × 10^6 hUCMSC intravenously, the vehicle group (n=10) received phosphate buffered saline (PBS) whereas the control group (n = 10) receive nothing. All injections were performed one day after injury into the tail veins of the rats. All cells were labelled with Brdu before injection. Evaluation of the neurological function of the rats was performed before and after injury using Neurological Severity Scores (NSS). The rats were sacrificed 6 weeks after TBI and brain sections were stained using Brdu immunohistochemistry.
Results: Statistically significant improvement in functional outcome was observed in the treatment group compared with the control group ($p < 0.01$). This benefit was visible 1 week after TBI and persisted for six weeks (end of trial). Histological analysis showed that hUCMSC were present in the lesion boundary zone at 6 weeks in all cell injected animals.
Conclusion: Rats injected with hUCMSC after TBI survive for at least six weeks and show functional improvemnt.

Keywords: Stem Cell, Injection, Brain Injury

Yakhteh Medical Journal, Vol 12, No 1, Spring 2010, Pages: 87-96

Introduction

Traumatic brain injury (TBI) influences people of all ages. The prevalence is greater in young adults and results in huge costs both for the individual and society. Until now there has been a shortage of neuroprotective therapies for neurotrauma in humans (1, 2). The pathophysiology and after-effects following TBI are very diverse in kind and nature (3), which is the most likely reason no universal treatment for TBI exists. Stem cells from different sources, such as peripheral blood, bone marrow and embryonic stem cells, have been used to induce angiogenesis, associated with local proliferation, differentiation and angiogenic cytokine production of implanted cells within ischemic tissues(4-7). Although the engraftment of transplanted cells and subsequent cytokine production in the targeted ischemic and traumatically injured organs are critically important for inducing angiogenesis and synaptogenesis, the complex cellular and molecular mechanisms involved in cell-based formation of new synapses and neurons are still poorly understood. Wharton's jelly (WJ) from the umbilical cord is a novel source of stem cells (8). Detailed immunohistochemical and ultrastructural examinations have found that cells from the WJ of umbilical cords possess characteristics of stromal cells. In recent studies, matrix cells from umbilical cord WJ have been shown to differentiate into neural-like cells both in vitro and in vivo (8, 9). In this study we have isolated a cell population derived from the WJ of human umbilical cords which was expanded in vitro and maintained in culture. We used this cell population to investigate migration and survival after intravenous transplantion of human umbilical cord matrix stem cell (hUCMSC) or WJ stem cell in TBI in rats.

Materials and Methods

Animal model

Adult male Wistar rats, weight 250 to 300 g, were used in this study. Animals were kept in a

room at 23°C ± 2, humidity 45% to 55% with a fixed 12-h artificial light period and allowed to eat and drink ad libitum. All animals were fed a standard rodent diet and received human care, as outlined in the guide for the care and use of laboratory animals. This study was approved by the Ethical Committee of Iran University of Medical Sciences (Tehran, Iran). A controlled cortical impact model in rat was used. Male Wistar rats (n = 30) were anesthetized by intraperitoneal injection with chloral hydrate 350 mg/kg bodyweight. Rectal temperature was controlled at 37°C ± 0.5°C with a feedback-regulated water-heating pad. A controlled cortical impact device (Fig 1) was used to induce the injury. Rats were placed in a stereotactic framee. 6-mm diameter craniotomies (Fig 1) were performed contiguous to the central suture, midway between lambda and bregma. The dura over the top of the cortex was kept unimpaired. Injury was induced by impacting the right cortex (ipsilateral cortex) with a piston containing a 5-mm diameter tip at the rate of 4 m/s and 2.5 mm of contusion (10). Animals were divided into three groups as follows:

Group 1 (10): Control group (Only TBI)
Group 2 (10): TBI + PBS
Group 3 (10): TBI + 2 × 10^6 hUCMSC

Culture of human umblical cord matrix stem cell

The human umbilical cord tissues were washed three times with Ca^{2+} and Mg^{2+}-free PBS. They were mechanically cut by scissors in a midline direction and the vessels of the umbilical artery, vein and outlining membrane were dissociated from the WJ .The jelly was then cut into pieces smaller than 0.5 cm^3, treated with collagenase type 1 (Sigma) and incubated for 14-18 hours at 37 °C in a 95% air/5% CO_2 humidified atmosphere. The explants then were cultured in DMEM containing 10% fetal calf serum (FCS) and antibioties at 37 °C in a 95% air/5% CO_2 humidified atmosphere. They were left undisturbed for 5-7 days to allow for migration of the cells from the explants.

The cellular morphology became homogenously spindle-shaped in cultures after 2-3 passages.

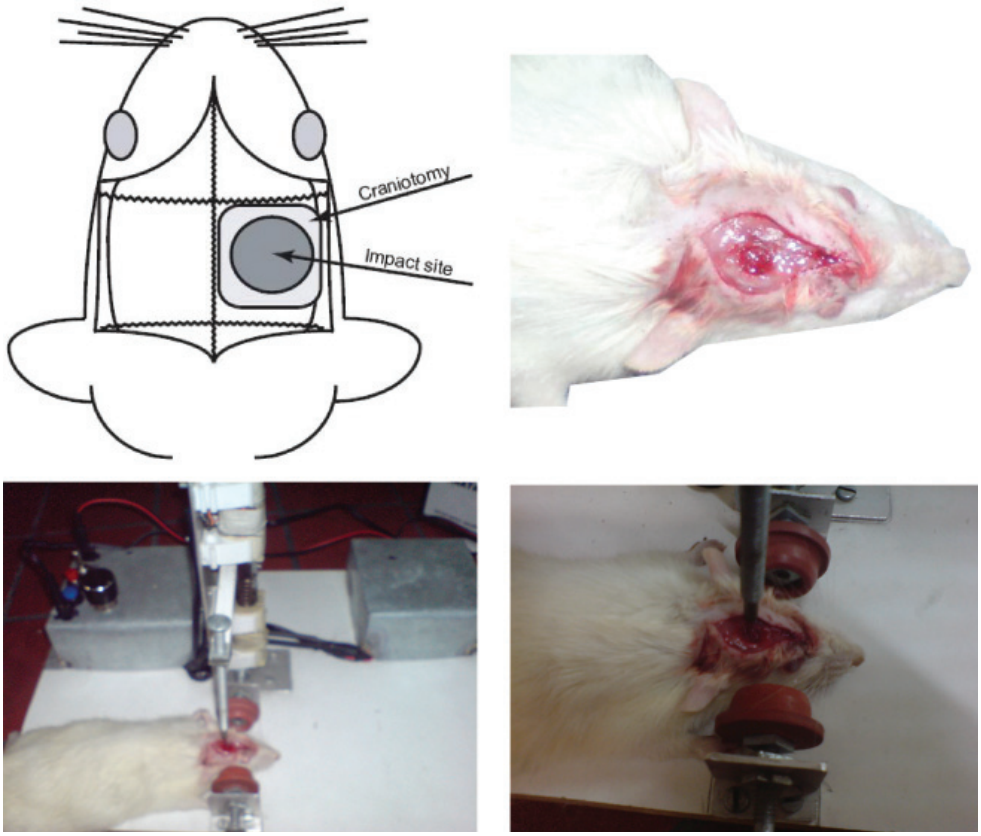

Fig 1: The CCI (controlled cortical impact) set-up consists of an piston causing a mechanical trauma in mice and results in cortical contusions, morphological and cerebrovascular effects, subdural and intra-parenchymal hematoma, edema, inflammation and changes in cerebral blood flow which clearly mimics human traumatic brain injury.

Alkaline phosphatase staining procedure

We used CHEMICON's Alkaline Phosphatase Detection Kit (Catalog number SCR004) hUCMSC cells were cultured at low to medium density for five days prior to analyzing AP activity; (according to our findings, five days of culturing spindle-shaped cells are optimal for good AP stain visualization). On day five the media were aspirated and the hUCMSC fixed with 4% Paraformaldehyde in PBS for 1-2 minutes. The fixative was then aspirated and the cells rinsed with 1 X Rinse Buffer (TBST: 20mM Tris-HCl, pH 7.4, 0.15 NaCl, 0.05% Tween-20). Reagents for Alkaline Phosphatase staining Naphthol/ Fast Red Violet Solution were prepared by mixing Fast Red Violet (FRV) with Naphthol AS-BI phosphate solution and water in a 2:1:1 ratio (FRV: Naphthol:water). Enough stain solution was added to cover each well (e.g. 0.5mL for each well in a 24-well plate) and plates were incubated in the dark at room temperature for 15 minutes. The staining solution was then aspirated and the wells rinsed with 1 X Rinse Buffer. Cells were then covered with 1 X PBS to prevent drying and the number of colonies counted.

Stem cell labeling and transplant

To identify cells derived from hUCMSC, 3 mg/ mL bromodeoxyuridine (BrdU) (Sigma) a thymidine analog and marker of newly synthesized DNA was added to the medium 72 hours before transplantation. Upon harvest, cells were isolated by treatment with 0.25% trypsin and 0.5 mM ethylenediaminetetraacetic acid (EDTA) at 37 °C for 10 minutes at room temperature. The digestion was stopped by adding FCS. Cells were then washed five times with PBS. Nucleated hUCMSC were counted using a cytometer to ensure adequate cell numbers for transplantation. For immunostaining hUCMSC were subcultured in chambered slides and more than 90% of hUCMSC were BrdU reactive. Approximately 2×10^6 hUCMSC in 200 µL PBS (n = 10) or control fluid 200 µL PBS (n=10) was slowly injected over a 5-minute period into each rat tail vein. Immunosuppressants were not used in animals in this study.

Brdu immunohistochemical assessment

The injured cerebral tissues (5mm) were cut into coronal paraffin blocks. Single immunohistochemical staining (11) was used to identify cells derived from hUCMSC. A series of 6-mm-thick sections at various levels (100-µm interval) were cut from this block and were analyzed by fluorescent microscopy. After deparaffinization, sections were placed in boiled citrate buffer (pH 6.0) in a microwave oven (650 to 720 W). After blocking in normal serum, sections were treated with the monoclonal antibody against BrdU in mouse (Sigma) diluted at 1:100 in PBS. After sequential incubation with rhodamine conjugated goat anti-mouse IgG (dilution 1:60; Chemicon), the secondary antibody was bound to the first antibody against BrdU. Cells derived from hUCMSC were identified by morphological criteria and by immunohistochemical staining with BrdU (the tracer) present in the nuclei of donor cells. Analysis of BrdU-positive cells was based on the evaluation of an average of 10 slides. All BrdU-reactive cells, with BrdU clearly localized to the nucleus, were counted throughout all 10 coronal sections (12).

Neurological functional evaluation

Neurological function (Table 1) in the rats was assessed using the neurological severity score (NSS). The NSS is composed of motor (muscle status, abnormal movement), sensory (visual, tactile and proprioceptive), reflex, and beam walking tests. In the severity scores for injury, one point is awarded for the inability to correctly perform the tasks or for the lack of a tested reflex. The higher the NSS score observed, the more severe the injury. The evaluation of all rats was started before TBI and performed after TBI at one week, and weekly thereafter. All measurements were performed by observers blinded to individual treatment (13).

Nissl staining method

This method is used for the detection of Nissl bodies in the cytoplasm of neurons on paraformaldehyde or formalin-fixed paraffin embedded tissue sections. The Nissl body will be stained purple-blue. This stain is commonly used for identifying the basic neuronal structure in brain and spinal cord tissue. After fixing the brain with 4% paraformaldehyde in 0.1M PB or 10% formalin paraffin sections of 20 µm were cut. These sections were deparaffinized with 2 or 3 10-minute changes of xylene, hydrated in 100% alcohol for 2×5 minutes, 95% alcohol for 3 minutes, 70% alcohol for 3 minutes and then rinsed in tap water followed by distilled water.

They were then stained in 0.1% cresyl violet solution for 3-10 minutes. Staining in warmed cresyl violet solution (37-50 ºC in an oven) can improve penetration and enhance even staining.

Table 1: The table shows a set of modified neurologic severity scores used to grade neurologi function

Moto tests	**Points**
Raising the rat by the tail	3
1 Flexion of forelimb	
1 Flexion of hindlimb	
1 Head moved more than 10° to the vertical axis within 30 s	
Walking on the floor (normal=0; maximum=3)	3
0 Normal walk	
1 Inability to walk straight	
2 Circling toward the paretic side	
3 Falling down to the paretic side	
Sensory tests	2
1 Placing test (visual and tactile test)	
2 Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)	
Beam balance tests (normal=0; maximum=6)	6
0 Balances test steady posture	
1 Grasps side of beam	
2 Hugs the beam and one limb falls down from the beam	
3 Hugs the beam and one limbs fall down from the beam, or spins on beam (>60 s)	
4 Attempts to balance on the beam but falls off (>40 s)	
5 Attempts to balance on the beam but falls off (>20 s)	
6 Falls off: no attempt to balance od hang on to the beam (<20 s)	
Reflexes absent and abnormal movements	4
1 Pinnar reflex (a head shake when the auditory meatus is touched)	
1 Corneal reflex (an eye blink when the cornea is lightly touched with cotton)	
1 Strartle reflex (a motor response to a brief noise from snapping a clipboard and paper)	
1 seizures, myoclonus, myodystony	
Maximum points	18

One points awarded for the inability to perform the task or for the lack of a tested reflex: 13-18=severe injury; 7-12=moderate injury; 1-6-mild injury.

It is particularly beneficial for thicker (20-50 um) sections. After staining, sections were rinsed quickly in distilled water and then differentiated in 95% ethyl alcohol for 2-3 minutes, checked by microscope for optimal results and then dehydrated in 100% alcohol for 2×5 minutes, cleared in xylene for 2×5 minutes and mounted with permanent mounting medium.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Duncan post-hoc test using SPSS 15.0. The data are presented as the Mean ± SD (standard deviation). P-values of less than 0.05 were considered to denote statistically significant differences and P-values <0.01 were defined as denoting highly significant differences. All P-values >0.05 were interpreted as representing no significant difference.

Results

Isolation, expansion and characterization of hUCMSC from human umbilical cord

After preparing the WJ primary cultures, adherently growing cells of spindle-shaped morphology were seen to migrate from the explants (Fig 2). Cells from WJ were fed and changed with culture medium twice weekly, and passaged as necessary. Freshly isolated cells (P0) principally displayed a fibroblast-like appearance over the first 3-4 days of culture.(Fig 2A) During the 2nd week, they typically appeared as slender cells with narrow cytoplasm and few lamellopodia (Fig 2B-F). After 12-14 days, they grew to 100% confluence (Fig 2G) and formed colonies (Fig 2H) and P1 cells resembled P0 cells.

We used alkaline phosphatase to detect stem cells and observed colonies express alkaline

phosphatase (red or brown stem cell colonies) (Fig 3). hUCMSC cell culture can be maintained either by harvesting the neurosphere-like cell clusters or by passage of preconfluent flat and spherical cells without apparent differences. We have maintained hUCMSC cultures for more than 50 population doublings and they continue to grow vigorously.

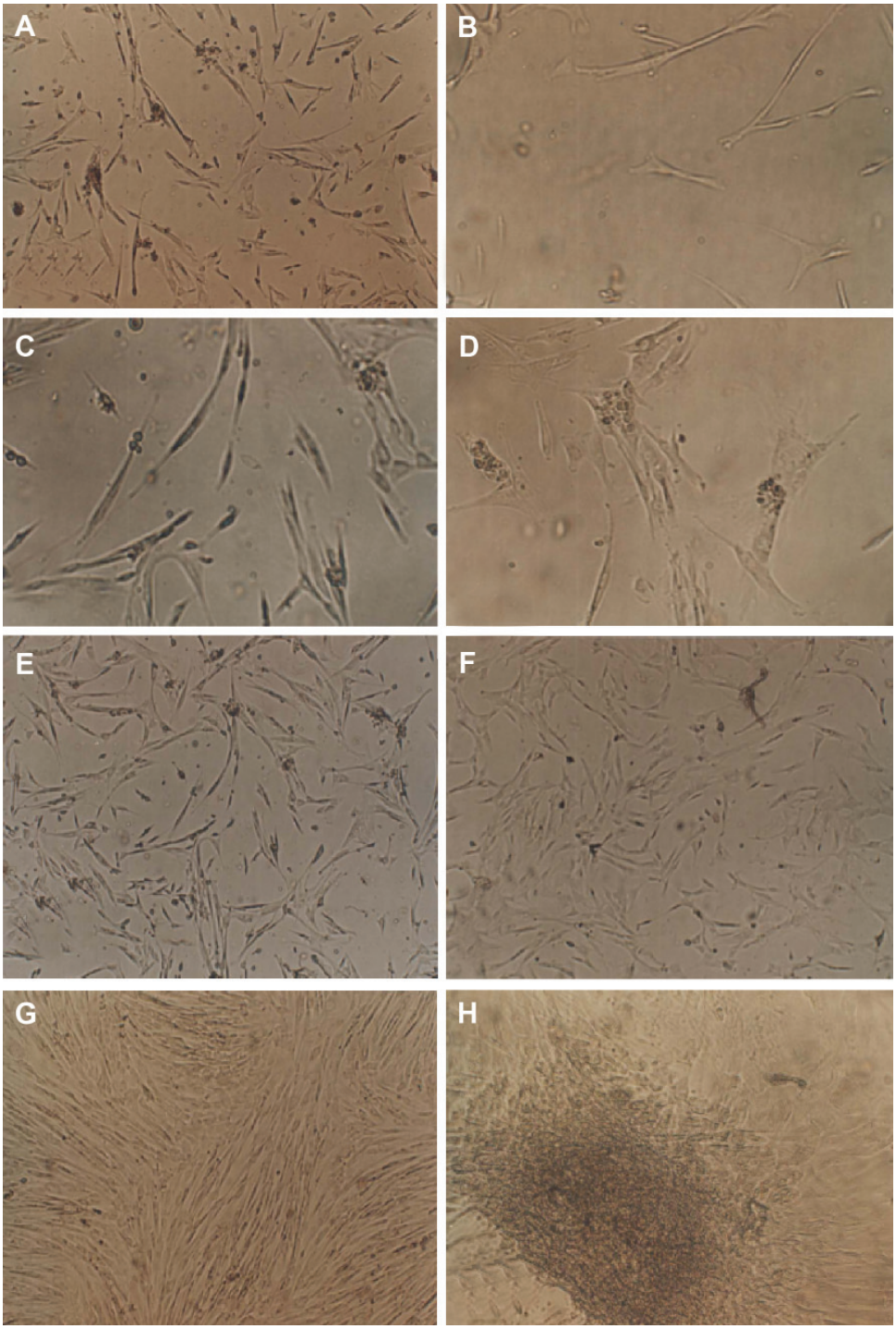

Fig 2: Pluripotential cells from the human umbilical cord matrix.(A-H) Appearance and growth of fibroblastoid cells or human umbilical cord matrix stem cell at passage 0 on days 3, 7(A-F), 12 (G) 14 (H) respectively. Briefly when hUCMSC cells initially grow outward from explants two morphologically distinct populations of cells are present: spherical or flat mesenchymal cells. When the cells become confluent, they form spherical colonies that remain attached to cells. These colonies resemble "neurospheres"

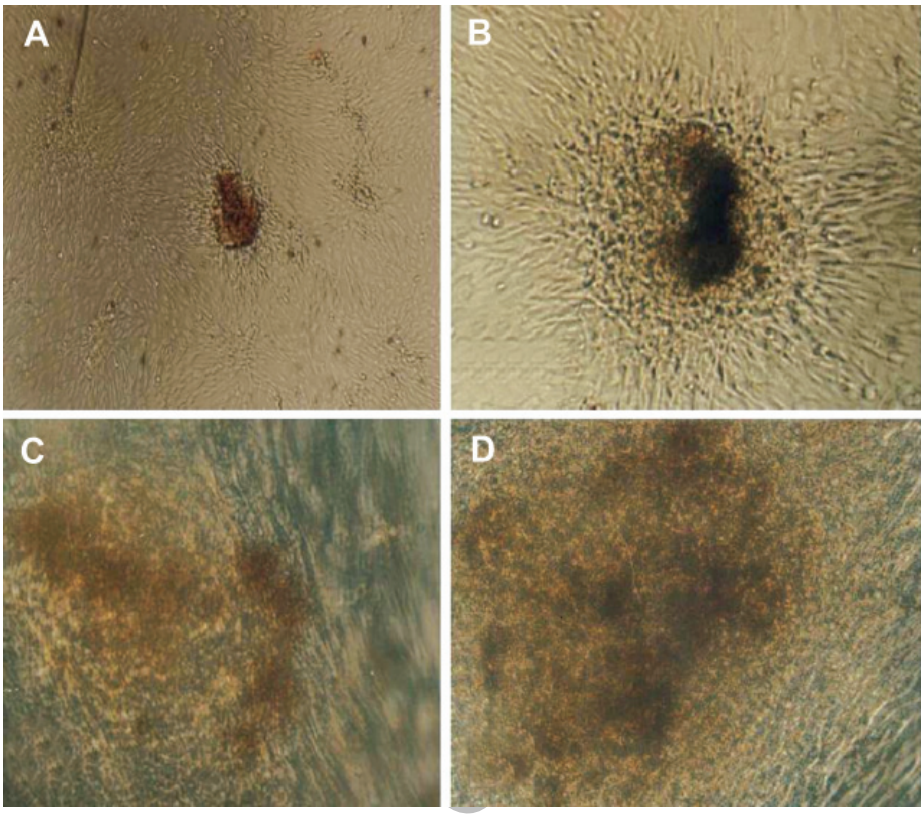

Fig 3: Human umblical cord matrix stem cell colony express alkaline phosphatase activity (A-D).

Table 2: Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by Duncan post-hoc test with SPSS 15.0. The data are presented as the Mean ± SD

Time Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	10.3 ± 0.483	9.4 ± 0.699	8.6 ± 0.516	7.1 ± 0.567	6.1 ± 0.567	5.5 ± 0.527
Sham	10.3 ± 0.483	9.4 ± 0.699	8.6 ± 0.516	7.1 ± 0.567	6.1 ± 0.567	5.5 ± 0.527
hUCMSC	9.2 ± 0.632	8.2 ± 0.918	6.6 ± 0.699	5.8 ± 0.632	4.9 ± 0.737	3.9 ± 0.737

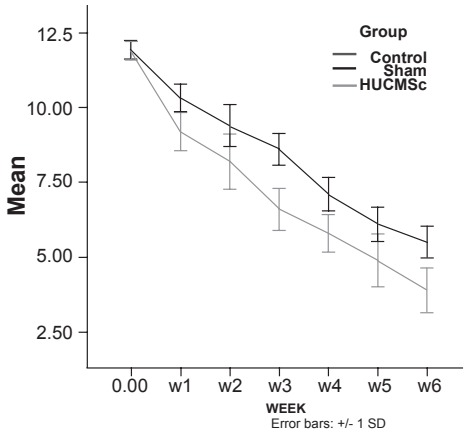

Fig 4: Results of behavioral functional tests (modified neurologic severity score [mNSS] test) before and after traumatic brain injury (TBI). Rats were treated to traumatic brain injury alone (Control) (n = 10) or were injected with cultured human umblical cord matrix stem cell (hUCMSC) (n = 10) or SHAM (PBS) (n = 10) 1 day after TBI. Significant functional recovery was detected in rats treated with hUCMSC compared with control and sham, p-value<0.01. The data are presented as the Mean ± SD. (W=Week)

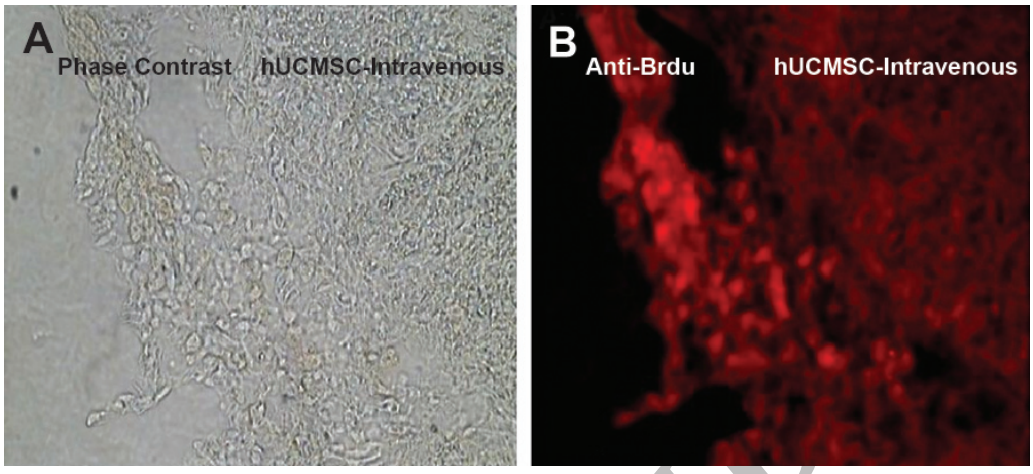

Fig 5: 42 days after traumatic brain injury 41 days after hUCMSC intravenous transplantation, cells derived from hUCMSC identified by rhodamin conjugated secondary antibody (B-red) distribute in the territory of the TBI (× 40).

Three cellular characteristics indicate that undifferentiated hUCMSC are a type of stem cell: (1) the number of passages through which they have been maintained in culture; (2) the fact that UCM cells are telomerase-positive; and (3) UCM cells make the receptor for stem cell factor, c-kit (5).

Neurological and motor function evaluation

Injury in the right hemispheric cortex of rats produced neurological functional problems, as measured by the NSS, which is a combination of motor, sensory, reflex and beam walking inspections. These rats exhibited high scores on motor, sensory, reflex, and beam balance tests in the early phase after injury (first week post injury). Recovery began in the end of first week and continued at all following appraisal times in both PBS-treated and hUCMSC groups. Motor function assessed by the NSS recovered faster than sensory and beam balance functions. NSS scores for all hUCMSC-treated groups were significantly below the NSS scores of the PBS-treated and control groups; p-value <0.01 betokening an amelioration of the functional consequence of injury (Table 2 and Fig 4).

Presence of brdu positive cells

The analysis of BrdU-reactive cells is based on the evaluation of an average of 10 histology slides per treatment animal taken from multiple areas of the ipsilateral hemisphere, including cortexes, striatum of the ipsilateral hemisphere. The massive bulk of BrdU-labeled cells were located in the traumatic core and its boundary zone. A small number of cells were discerned in the contralateral hemisphere. In summary, hUCMSC-treated traumatic rats exhibited significantly increased numbers of BrdU immunoreactive cells in their traumatic core compared with control and saline injected traumatized rats (Fig 5).

Discussion

We have determined that intravenous injection of 2×10^6 hUCMSC one day after TBI significantly ameliorates functional impairment compared with non-treated rats. Morphological analysis of the tissue reveals that BrdU-labeled hUCMSC are more likely to enter into damaged brain than into contralateral non traumatic brain. What are likely to be the mechanisms or factors that amplify the reduced impairment observed with hUCMSC transplantation after TBI Wharton Jelly is composed of myofibroblast-like stromal cells (14). Myofibroblasts are important in growth, development and repair and are found throughout the body (15). As previously shown, new blood vessel growth was thought to be exclusively dependent on the growth of endothelial cells (16). However, the exact mechanism that leads to angiogenesis or collateral circulation of arteries after stem cell transplantation is not yet known. A recent study pointed out not only endothelial cells may be essential (17). Many reports have proposed that the “injured” brain might specifically draw in stem cells or that stem cells might “home in” on the injured brain (18, 19). Recently Stumm et al. demonstrated that focal cerebral ischemia causes an extension in SDF-1 expression in regions proximate to the infarcted area (20). SDF-1 is a CXC

chemokine constitutively produced by bone marrow stromal cells and is a vigorous chemoattractant for stem cells. In one study (21) a lesion-induced upregulation of endothelial SDF-1 (RT-PCR results) and the arrival of increased bis-benzimide-labeled CXCR4-expressing cells in the ischemic hemisphere might imply that cerebro-endothelial SDF-1 is a chemo-attractant for hUCMSC. Additionally, bis-benzimide-labeled stem cells, co-stained with a specific marker for doublecortin (22) and transplanted in the striatum were detected to migrate into the penumbra of the ischemic cortex 1 month after transplantation. This result implies that, after traumatic brain injury, hUCMSC were commanded toward sites of brain injury, where they could be involved in brain repair and functional recovery. Our data also demonstrate that traumatic brain tissue persuaded migration and homing of hUCMSC into damaged tissues. An SDF-1/CXCR4 interaction may be directly involved in vascular remodeling, angiogenesis and neurogenesis , as a result of that alleviating traumatic symptoms. In addition, hUCMSC migrating to the traumatic hemisphere could produce local chemical gradients and localized chemokine accumulation, dictating a directional response in endothelial, neuronal and glial progenitor cells (23). As a logical result of this autocrine regulatory pathway, endothelial and neuronal progenitor cells could mobilize and fuse with each other, a step required for subsequent formation of a structured network of branching vessels and neurons (24). In this study significant functional recovery was found 7 days after transplantation of hUCMSC. Thus, we sugest that the repair mechanism after stem cell transplantation in the traumatic brain might be separated into two stages of "growth factor" and "cell repair", as follows. Initially, it is improbable that these transplanted hUCMSC are incorporated into the traumatic tissue and make viable connections within days after transplantation due to the short time period. Instead, it seems likely that during the "growth factor phase" transplanted hUCMSC interact with the host CNS and lead to the production of cytokines or growth factors that then elevate the functional recovery of the remaining survival neurons and decrease apoptosis at the traumatic boundary zone. This hypothesis is based on the fact that growth factors and cytokines are the molecular signals that govern cell survival, proliferation and differentiation (25) and that exogenously administered neurotrophic growth factors have been shown to restrain the breadth of acute ischemic neural injury and reinforce functional recovery after stroke (26). It has been proved (21) that mRNA levels of neurotrophic factors including SDF-1, BDNF and GDNF increased significantly 7 days after transplantation in hUCMSC-treated rats' brains compared to controls. Therefore we suggest that the grafted hUCMSC and their migration into the injured tissue may make available a neurotrophic factor production source and interact with the host brain, resulting in hUCMSC and parenchymal cells producing supplemental neurotrophic factors which may be involved in functional reorganization of the traumatic brain. hUCMSC may, as a result, be directly associated in promoting plasticity of traumatically damaged neurons or in instigating glial cells to secrete neurotrophins (BDNF and NGF) (27). It has been shown (9) that injection of porcine UCM cells into rat brain do not evoke an immune response. It has been described (8) that human UCM cells have the facility to differentiate into a neuronal phenotype in vitro. It has been shown that rat UCM cells are myofibroblast-like stem cells, similar to those separated from porcine and human umbilical cord. They express markers similar to those found in the adult bone marrow stromal stem cell (SMA and vimentin) and embryonic stem cells (Oct-4) (28). These findings suggest that the UCM is an profuse, easily obtainable source of primitive Oct-4+ stem cells that might be intermediary between embryonic and adult stem cells. It has been shown that unlike embryonic stem cells, hUCMSC cells do not form teratomas, nor do they evoke a detectable immune response (29). Mesenchymal stem cells from cord blood, while having many of the same characteristics as the hUCMSC cells, are less profuse and might be less primordial than those found in hUCMSC. Because of these attributes, hUCMSC cells might be a better substitute for embryonic, bone marrow stromal, or umbilical cord blood cells for cell-based therapies. It shown the presence of Oct-4+ RUCM (rat umbilical cord matrix cells) in the site of ischemia stimulates and increase the speed of the proliferation and recruitment of the endogenous neuronal stem cells, including re-entry of quiescent stem cells, into the rescue effort (28). Other probabilities comprise the production of extracellular surroundings that reinforce and aid restitution of the intrinsic ability of the brain tissue to self-repair.

Conclusion

Given the restricted accessibility of organs for

transplantation, cell transplant is an innovative and promising treatment for the repair of injured organs, such as brain. Nevertheless, although results in animal models and some introductory clinical trials appear to be hopeful, it is too early to make any definitive judgment as to the usefulness of such therapy. Furthermore, a number of essential questions remain: what is the best type of cells for and how many cells do we need to inject to be forcible? what is the best time (acute or chronic) for administration of cells and what is the best method for cell delivery? We are undoubtedly at the starting-point of a new and challenging field. New animal studies and scrupulously planned phase 1 clinical trials are needed to try to answer some of these questions and many new ones that will follow.

Acknowledgments

This study was supported financially by the Research Authority of Iran University of Medical Sciences. The authors wish to acknowledge Dr. Mohammad Taghi Joghataei , Head of Department of Anatomy for his help, and Masoomeh Bakhshayesh, technician of cellular and molecular research center for her cooperation and help. There is no conflict of interest in this article.

References

1. Dutton RP, McCunn M. Traumatic brain injury. Curr Opin Crit Care. 2003; 9: 503-509.
2. Gunnarsson T, Fehlings MG. Acute neurosurgical management of traumatic brain injury and spinal cord injury. Curr Opin Neurol. 2003; 16: 717-723.
3. Rosomoff HL, Kochanek PM, Clark R, DeKosky ST, Ebmeyer U, Grenvik AN, et al. Resuscitation from severe brain trauma. Crit Care Med. 1996; 24: 48-56.
4. Jackson KA, Majka SM, Wang H. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest. 2001; 107: 1395-1402.
5. Kocher AA, Schuster MD, Szabolcs MJ. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med. 2001; 7: 430-436
6. Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, Langer R. Endothelial cells derived from human embryonic stem cells. Proc Natl Acad Sci USA. 2002; 99: 4391-4396.
7. Strauer BE, Brehm M, Zeus T. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation. 2002; 106: 1913-1918.
8. Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D. Matrix cells from Wharton's jelly form neurons and glia. Stem Cells. 2003; 21: 50-60.
9. Weiss ML, Mitchell KE, Hix JE. Transplantation of porcine umbilical cord matrix cells into the rat brain. Exp. Neurol. 2003; 182: 288-299.
10. Dixon E, Clifton G, Lighthall JW. A controlled cortical impact model of traumatic brain injury in rat. J Neurosci Methods. 1991; 39: 253-262.
11. Li Y, Powers C, Jiang N, Chopp M. Intact injured, necrotic and apoptotic cells after focal cerebral ischemia in the rat. J Neurol Sci. 1998; 156: 119-132.
12. Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentategyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci. 1996; 16: 2027-2033.
13. Chen Y, Lomnitski L, Michaelson DM, Shohami E. Motor and cognitive deficits in apolipoprotein E-deficient mice after closed head injury. Neuroscience. 1997; 80: 1255-1262.
14. Kobayashi K, Kubota T, Aso T. Study on myofibroblast differentiation in the stromal cells of Wharton's jelly: expression and localization of alpha-smooth muscle actin. Early Hum Dev. 1998; 51: 223-233.
15. Powell DW, Mifflin RC, Valentich JD. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Physiol. 1999; 277: C1-C9.
16. Schaper W, Ito WD. Molecular mechanisms of coronary collateral vessel growth. Circ Res. 1996; 79: 911-919.
17. Heil M, Ziegelhoeffer T, Pipp F. Blood monocyte concentration is critical for enhancement of collateral artery growth. Am J Physiol Heart Circ Physiol. 2002; 283: H2411-H2419.
18. Chen S T, Hsu C Y, Hogan E L, Maricq H, Balentine JD. A model of focal ischemic stroke in the rat: reproducible extensive cortical infarction. Stroke. 1986; 17: 738-743.
19. Petersen BE, Bowen WC, Patrene KD. Bone marrow as a potential source of hepatic oval cells.Science. 1999; 284: 1168-1170.
20. Stumm J, Rummel V, Junker C, Culmsee M. Pfeiffer A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. J Neurosci. 2002; 22: 5865-5878.
21. Ding DC, Shyu WC, Ching MF, Lin SZ, Chang YC, Wang HJ, et al. Enhancement of neuroplasticity through upregulation of β1-integrin in human umbilical cord-derived stromal cell implanted stroke model.Neurobiol Dis. 2007; 27: 339-353.
22. Jiang W, Gu W, Brannstrom T. Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. Stroke. 2001; 32: 1201-1207.
23. Yamaguchi J, Kusano KF, Masuo O. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation. 2003; 107: 1322-1328.
24. Chen J, Li Y, Wang L, Zhang Z. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke. 2001; 32: 1005-1011.
25. Yoshimura S, Takagi Y, Harada J. FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. Proc Natl Acad Sci USA. 2001; 98: 5874-5879.
26. Ay I, Sugimori H, Finkelstein SP. Intravenous basic fibroblast growth factor (bFGF) decreases DNA fragmentation and prevents downregulation of Bcl-2 ex-

pression in the ischemic brain following middle cerebral artery occlusion in rats. Brain Res Mol Brain Res. 2001; 87: 71-80.
27. Hefti F. Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. J Neurosci. 1986; 6: 2155-2162.
28. Sachiko Jomura, Mark UY, Kathy mitchel. Potential Treatment of Cerebral GlobalIschemia with Oct-4+ Umbilical Cord Matrix Cells. Stem Cells. 2007; 25: 98-106.
29. Medicetty S, Bledsoe AR, Fahrenholtz CB. Transplantation of pig stem cells into rat brain: Proliferation during the first 8 weeks. Exp Neurol. 2004; 190: 32- 41.