Original Article

Co-transplantation of Schwann and Bone Marrow Stromal Cells Promotes Locomotor Recovery in the Rat Contusion Model of Spinal Cord Injury

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Received: 12/Aug/2009, Accepted: 15/Oct/2009

Abstract

Objective: Previous studies have shown that transplantation of bone marrow stromal cells (BMSCs) into the contused spinal cord improves functional recovery and that administration of Schwann cells (SCs) after spinal cord injury (SCI) facilitates axonal regeneration. Although the efficacy of these treatments have been proven, when used individually, their resulting number of regenerated axons is small and locomotor recovery is modest; therefore, we decided to research whether co-transplantation of these cells can improve the outcome.

Materials and Methods: Adult male Wistar rats (n=56), each weighting 250-300 grams were used. BMSCs and SCs were cultured and prelabeled with BrdU and 1,1' dioctadecyl 3,3,3',3' tetramethylindocarbocyanin perchlorate respectively. Contusion model of SCI was performed at the T8-9 level using NYU device (New York University device). The rats were divided into seven groups, each consisting of 8 animals. These groups included: a control group, three experimental groups and three sham groups. In the control group, only a laminectomy was performed. The three experiment groups were the BMSC, SC and co-transplant groups, and 7 days after injury, they received intraspinal BMSCs, SCs and the combination of BMSCs & SCs respectively. The sham groups received serum in the same manner. Locomotion in the groups was assessed using the basso, beatie and bresnahan (BBB) test at 1, 7, 14, 21, 28, 35, 42, 49 and 56 days after SCI.

Results: More significant improvement was observed in the BBB scores of the co-transplant group ($p<0.05$) in comparison with BMSC and SC groups.

Conclusion: This study shows that co-transplantation of BMSCs and SCs may provide a powerful therapy for SCI and become required for the development of combinatory treatment strategies in the future.

Keywords: Bone Marrow Stromal Cell, Schwann Cell, Transplantation, Spinal Cord Injury, Functional Recovery

Yakhteh Medical Journal, Vol 12, No 1, Spring 2010, Pages: 7-16

Introduction

Spinal cord injury (SCI) is one of the most disabling diseases leading to progressive tissue damage and causing significant functional deficits in young people (1). SCI has various physical and psychological effects on patients; therefore, adequate and immediate therapeutic procedures for its treatment are necessary. Since the central nervous system (CNS) has a limited regeneration capacity, cell transplantation is one of the most effective methods to repair its injured axons (2). The aims of cellular transplantation after SCI include: (a) replacing damaged or dead cells (b) bridging the resulting cavities or cysts (c) providing a suitable environment to induce axonal regeneration and (d) promoting neovascularization.

During the last decade, several cell types have been used for transplantation including embryonic stem cells (ESCs) (3, 4), olfactory ensheathing cells (OECs) (5, 6), neural stem cells (NSCs) (7), Schwann cells (SCs), bone marrow stromal cells (BMSCs) (8, 9) and macrophages (10). BMCs are

one of the rich sources of stem cells; they can be easily isolated and are therefore suitable for autologous transplantation.

BMSCs have the ability to migrate to sites of lesion and improve functional recovery (11). Different studies indicate that BMSCs, after transplantation in mice and rats, can differentiate into adipocytes, chondrocytes, osteocytes, (12) oligodendrocytes, less frequently to neurons, and are able to express the markers of these cells (13). They can promote injured tissue repair by reducing cavity formations caused by tissue necrosis at sites of lesion (14). BMSCs create a suitable axonal growth environment through the expression of growth factors such as neurotrophins (15, 16). They can also improve vascularization (16) leading to damaged tissue repair. Furthermore, Akiyama et al. reported that injection of BMSCs into injured areas leads to myelination of demyelinated axons (11).

Another cell type used for repair of the injured spinal cord is the Schwann cell (SC). SCs can myelinate and ensheath axons and provide physical support for axonal growth when they are injected into a spinal cord lesion area (17, 18).

SCs produce neurotrophic factors and cell adhesion molecules leading to axonal growth promotion (19). They also suppress cavity formation when transplanted into injured spinal cords (20, 21). Studies have shown that SC transplantation significantly improves tissue sparing and results in an increase in the number of myelinated axons in implantation areas (21). It has been reported that SC injection into a contused spinal cord promotes myelination and regeneration of supraspinal axons and therefore improves locomotor recovery (21). The advantage of the SC compared to other cells commonly used in cell therapy is the superior ability of this cell to myelinate the demyelinated axon (22).

Several studies have reported that transplantation of BMSCs in the contused spinal cord of rats improves locomotor recovery and intraspinal administration of SCs facilitates axonal regeneration after SCI. Although the efficacy of these methods has been proven, when used individually, they result in a small number of regenerated axons, and a modest locomotor recovery (21). Since a combination-therapy strategy seems more promising, we decided to study whether co-transplantation of these cells can improve treatment outcome.

Materials and Methods

Animals

Adult male Wistar rats (n=56, Pasteur Institute, Tehran) each weighing 250-300 grams were used. All study procedures, as well as the animals, were approved by the Ethical Committee on Animal Experiments of the Research Council of Iran University of Medical Sciences (Tehran, Iran), and are in agreement with the Iranian National Institute of Health guidelines for the use of live animals.

BMSC Isolation

Bone marrow was isolated in sterile conditions from 8 week-old male Sprague Dawley rats weighing 250-300 grams as described in detail by Azizi et al. (23). Briefly, the rats were administered with an overdose of pentobarbital and their tibia and femurs were excised; both ends of each bone were cut, and the marrow was aspirated with 5ml MEMα (Sigma-Aldrich) with a 25-gauge needle. The suspension was centrifuged at 800 rpm for five minutes and the supernatant was removed. The marrow cells were suspended in 10ml of Alpha-MEM cultured in Alpha MEM supplemented with 10% fetal bovine serum (FBS), 2ml glutamine, 100 U/ml penicillin (Sigma-Aldrich) and 100 U/ml streptomycin (Sigma-Aldrich).

After 48 hours, the non-adherent BMSC cells were removed by replacing the medium. When the adherent cells had reached 80% confluency, they were lifted after a five minute exposure to a 0.25% trypsin / 1 mM EDTA (Sigma-Aldrich) containing solution at 37°C; they were then passaged into four subcultures for further incubation (Fig 1).

BMSC labeling

The cells were labeled with a 3 μg/ml bromodeoxyuridine (BrdU) solution added to the incubation medium 3 days prior to transplantation.

SC isolation

SCs were obtained from the sciatic nurve of 250-300 gram, ten-week old, adult female Wistar rats as described in 1991 by Morrissey et al. (24). Briefly, the rats were anesthetized with a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg). The left sciatic nerve of each animal was exposed and transected at the greater sciatic notch to allow for Wallerian degeneration. After 7 days, the animals were administered with overdose of pentobarbital, following which, 20 mm of the distal segment of their transected nerves were resected and placed in a dish containing DMEM (Sigma-Aldrich).

Under sterile conditions and using a dissecting microscope, the plural form of epineurium were removed with fine forceps; the nerves were then washed three times with phosphate buffered saline (PBS), transferred to a dish containing 10% fetal bovine serum (FBS) (Sigma-Aldrich), 1.25 IU/ml dispase (Sigma-Aldrich) and 0.05% collagenase

type IA (Sigma-Aldrich) and incubated for three hours. Then, the nerve cell suspension was centrifuged at 800 rpm for five minutes, the supernatant was discarded, and the pellet was washed with the medium and resuspended in DMEM supplemented with 10% FBS, 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma-Aldrich).

Cells in the suspension were counted with an hemocytometer and placed in 25 cm^3 flasks and incubated in 5% CO_2 at 37°C in a medium containing 10% FBS. After 2 days, non-adherent cells were removed by replacing the medium and the adherent cells were allowed to reach their confluent state. Once the adherent cells reached 80% confluency, the medium was replaced with 0.25% trypsin and 1mM EDTA (Sigma-Aldrich) and incubated at 37°C for five minutes.

Cell dissociation from the substrate was monitored under an invert microscope (Olympus 1×70, Japan) unit, and the maximum number of cells was lifted. The cell suspension was transferred to a 15 ml testtube where 10 ml PBS was added, and then centrifuged. The resulting supernatant was removed and replaced with a complete medium, then transferred to two 25 cm^3 flasks. The cells were subcultured once a week.

SC purification

After the Schwann cells were enzymatically stripped from the sciatic nerve thru incubation in collagenase and dispase, a considerable population of fibroblasts remained within the preparation. In order to remove the fibroblasts form SCs in the culture, the culture was incubated in antimitotic cytosine arabinoside (Ara-c, 1µl/ml, 5mM, Sigma) Sigma-Aldrich) 24 hours after the cells were first isolated (26, 27). After 5 to 7 days, ara-C had removed the majority of fibroblasts (Fig 4 A).

SC amplification

When SCs are cultured alone *in vitro*, cell division occurs extremely slowly, but may be stimulated by mitogens. In order to encourage the proliferation of SCs, the culture was incubated in forskolin (2µM, Sigma-Aldrich) (26).

SC labeling

The cells were labeled using the fluorescent lipophilic tracer 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanin perchlorate ('Dil';DilC18(3)) (Sigma-Aldrich) prior to transplantation. For labeling, with 1×10^6 cells/ml was resuspended in MEM Alpha, and of 5µl/ml 'Dil';DilC18 (3) was added. After incubation for 20 minutes at 37°C with 5% humidified CO_2, the cells were centrifuged for 5 minutes and washed twice with PBS; they were then resuspended in PBS for transplantation (28).

Spinal cord injury model

For spinal cord injury (SCI) simulation, female adult Wistar rats, each weighing 250-300grams, were used. The animals were anesthetized using ketamine (80 mg/kg) and xylazine (10 mg/kg). They were then placed prone on an operating table covered with a warming blanket. After shaving and prepping with Betadine, an incision was made over the rats mid-thoracic region. Laminectomy was performed at the T8-T9 level of the spinal cord. A standard spinal cord contusion was made using the New York University (NYU) weight-drop device.

A 10 gram metal rod with a 2 mm diameter was dropped from a height of 12.5 mm onto the exposed spinal cord at the T8 level causing a moderate contusion. After wound closure, the one-week postoperative care included manual bladder expression twice per day, administration of Ringer's solution to avoid dehydration (2 ml, IP) and administration of gentamicin (0.8 mg/100g, IP). Analgesia was achieved using buprenorphine (0.1 mg/kg) for 2 days after surgery. Passive mobilization of the hind legs was also performed 15 minutes daily for one week after surgery.

Histology

Four weeks post spinal cord injury, 4 animals were deeply anesthetized with sodium pentobarbital (100mg/kg, IP) and were transcardially perfused with 4% paraformaldehyde in 0.1mol/L PBS, pH 7.4. Their tissues were cryoprotected overnight in 30% sucrose; then a 1.5 cm segment of spinal cord containing the zone of injury was removed, embedded in optimal cutting temperature (OCT) and cut transversely into 8µm serial sections. These sections were stained with cresyl violet for the study of their general morphology.

Transplantation procedure

The rats were randomly divided into 7 groups: (1) a control group (n=8) in which only a laminectomy was performed; (2) a BMSC group (n=8) which received (3×10^5 BMSCs) intraspinaly and (3) its sham group (n=8) in which normal salin serum was injected in the same way; (4) an SC group (n=8) which received (3×10^5 SCs) and (5) its sham group (n=8) in which normal salin serum was injected in the same way; (6) a co-transplant group (n=8) which received 3×10^5 BMSCs and 3×10^5 SCs and (7) its sham group (n=8) in which normal salin serum was injected in the same way.

BMSCs were resuspended at a concentration of 30000 cells per μL. Seven days after SCI, the rats were anesthetized, the contusion site was reexposed, and the cell transplantation was performed as follows:
The suspension of BMSCs was aspirated using a Hamilton syringe with a sterile 30 gauge needle; the needle was then changed to a customized needle (110μm internal diameter), and the syringe was attached to a microinjector (model 780310). A small opening was made in the dura at the injection site. The customized needle was then inserted into the spinal cord at the midline at 1 to 1.5 mm depth, and a 10μL of the cell suspension was injected over 2 minutes at a distance of 1mm rostrally and then 1mm caudally from the site of injury. The needle was left in place for 2 minutes after injection before it was withdrawn to minimize cell leakage. The SCs and combination of BMSCs and SCs were likewise transplanted into the spinal cord. After each transplantation session, a sample of BMSCs and SCs from the Hamilton syringe was injected onto a slide and stained with trypan blue to assess cell viability.

Behavioral assessment
Locomotor activity was evaluated using the open-field test to assess locomotor ability during five minutes. One animal at a time was allowed to move freely inside a circular plastic tray (90 cm diameter × 24 cm wall height).
Two independent examiners observed the hindlimb movements of each rat and scored its locomotor function according to the Basso, Beattie, and Bresnahan scale (BBB scale) ranging from 0 (paralysis) to 21 points (normal gait). The final score for each animal was the mean value of both examiners scores. During the open-field activity, the animals were also video monitored with a digital camera.
Functional tests were performed before the injury and transplantation, as well as weekly for 8 weeks after transplantation. Significant differences were examined using repeated measures analysis of variance (ANOVA) followed by Tukey's test as post-hoc analysis. All values are given as mean ± SD.

Immunohistochemistry
Eight weeks after transplantation, the rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, IP) and were transcardially perfused with 4% paraformaldehyde (0.1 M phosphate buffer, pH 7.4).
Their tissues were cryoprotected in 30% sucrose overnight, and a segment of the spinal cord 1 cm in length from each side of the injury site was removed , embedded in OCT and parasagitally cryosectioned into 8μm serial sections.

BrdU immunohistochemistry
BMSCs labeled with BrdU prior to transplantation were identified according to the following procedure (29):
The sections were incubated in 50% formamide (Merck, Germany), 2×SSC (Standard Sodium Citrate: 0.3M NaCl and 0.03M sodium citrate) at 65°C for 2 hours , washed for 10 minutes with 2× SSC at room temperature then incubated in 2N HCl (Merck, Germany) at 37°C for 30 minutes. They were then rinsed in 0.1 M boric acid (Merck Germany) for 10 minutes, whashed in PBS and incubated with mouse anti-BrdU monoclonal antibody (Sigma-Aldrich) at 4°C overnight.
After rinsing 3 times in PBS for 10 minutes, the sections were incubated overnight in the dark at 4°C with fluorescein isothiocyanate (FITC)-conjugated secondary antibody (1: 100) (29). They were then washed in PBS, covered with a coverslip, and were studied under fluorescence microscope (Olympus AX70).

Immunohistochemistry for S100
To identify and evaluate the Schwann cell purity, immunoassaying of S100 was performed as follows: After the sections were washed 3 times in PBS for 6 minutes, they were incubated for 30 minutes in a blocking solution (1x PBS / 0.1% Triton X-100 / 2% normal goat serum), then incubated overnight at 4°C with cell marker primary antibody (rabbit anti-S100, code No: Z0311, Dako Cytomation). After rinsing 3 times in PBS, they were incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibody in the dark at room temperature for 60 minutes. The sections were then mounted onto gelatin-coated glass slides and analyzed under the fluorescence microscope.

Statistical Analysis
Statistical comparisons between the groups were carried out by using repeated measures analysis of variance (ANOVA) followed by Turkey's test as the post-hoc analysis.
Statistical analysis was performed using SPSS version 15. A p-value of <0.05 was accepted as statistically significant and all data was presented as mean ± SD.

Results

Histology
Four weeks after the contusion injury, observation of sections stained with cresyl violet revealed the formation of several differently sized vacuoles and cystic cavities at the site of injury. The cyst formation is due to the death of neurons, interneurons and glial cells after SCI.

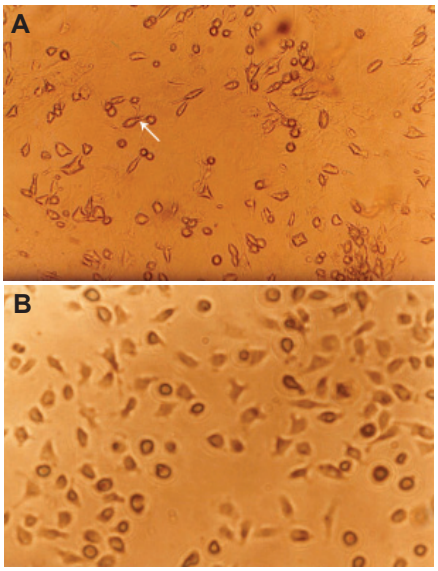

Fig 1: Bone marrow stromal cell culture in different stages. The morphology of bone marrow stromal cell in early (A) and late (B) stage of subculture.

Fluorescence microscopy (Fig 3) reveals that BrdU-positive BMSCs, transplanted at the site of injury, survived and reorganized around the cavity center. In Fig 4B fluorescence microscopy shows the presence of S-100-positive cells at the site of injury confirming that intraspinaly transplanted SCs survived for a long time.

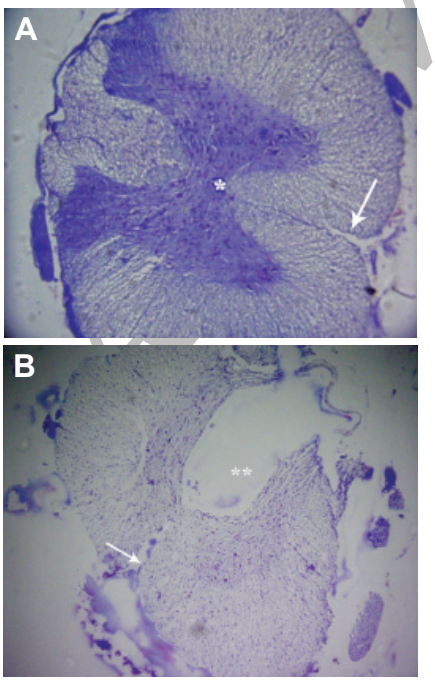

Fig 2: A) Cresyl violet stained 8µm thick cross section of spinal cord segments T8-9 of the control group. The star presents central canal, the arrow indicates median fissure. B) Cresyl violet stained 8µm thick cross section of the injury site 4 weeks after SCI. The stars present cystic cavities, the arrow indicates median fissure.

Immunohistochemistry findings

Immunohistochemistry findings confirmed the presence and viability of transplanted cells at the area of lesion. Eight weeks after transplantation, the immunohistochemistry results showed that transplants of BMSCs and SCs at the site of injury survived.

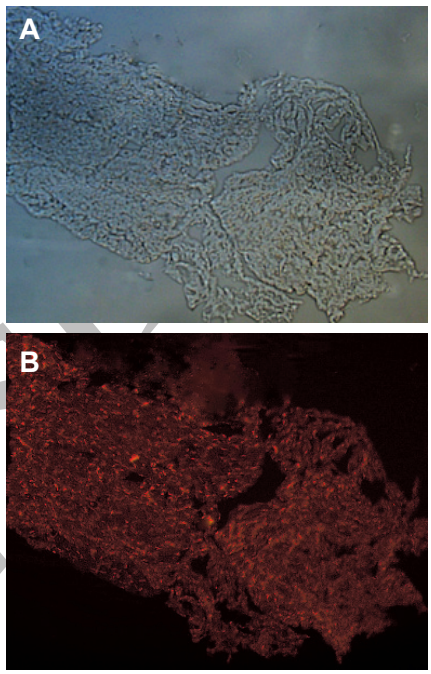

Fig 3: Cross-section immunohistochemical findings 8 weeks after transplantation of BMSCs. (A) Spinal cord tissue with site of injury is shown using a phase contrast microscope; star indicates the site of injury. (B) The arrow indicates BrdU-positive bone marrow stromal cells (dark red) which were present in the injured spinal cord after intralesional transplantation. The star indicates the site of injury.

Functional analysis

Prior to SCI, rats in all 3 experimental groups were assigned a BBB score of 21 points. One day after SCI, the contused rats demonstrated considerable loss of hindlimb locomotor function with no movement and BBB scores of 0-1 points.

During the first postoperative week, four of seventy rats in the BMSC group and three rats in both the SC and co-transplant groups died and were excluded from the study. Among the remaining rats (n=60), two in the BMSC group, and one in each of the SC and co-transplant groups showed hindlimb autophagia and were also excluded. Therefore, fifty six rats were assessed by BBB scoring.

On the following days, the BBB scores increased considerably in all groups; for example on the 14th post operative day (POD), the mean (± standard deviation) BBB scores were 8.12 ± 1.12 in the sham groups, 10.25 ± 1.28 in the SC group, 10.62 ± 1.30 in the BMSC group and 10.75 ± 1.16 in the

co-transplant group.
From weeks 3-8, the animals in the SC, BMSC and co- transplant groups exhibited a progressive increase in their hindlimb movements, especially when compared to the sham group ($p<0.05$) (Fig 5).
On the 7th and 8th weeks, rats in the co-transplant group improved their walking skills more significantly compared to the SC and BMSC groups. Moreover, animals in the co-transplant group displayed consistent weight-supported plantar stepping and demonstrated consistent fore-and hindlimb coordination, whereas the animals of SC and BMSC groups showed modest fore-and hindlimb coordination.
The control group animals had BBB scores of 21 during all stages of the study.
The 8th-week average BBB scores of animals in the sham, SC, BMSC and co-transplant groups were: (11.12 ± 1.12), (13.5 ± 1.06), (14 ± 0.75) and (15.87 ± 0.83) respectively (Fig 5).
Statistical analysis revealed significant differences between the experimental and control groups, between the experimental and sham groups and between the co-transplant and SC and BMSC groups ($p<0.05$); in contrast, the statistical difference between SC and BMSC groups was not significant ($p>0.05$) (Fig 5).

Discussion

This study indicates that the co-transplantation of BMSCs and SCs is more neuroprotective and improves BBB scores and axonal regeneration compared to transplantation of BMSCs and SCs individually.
Our histological findings confirmed that the animals used in this study underwent a contusion injury (Fig 2B).

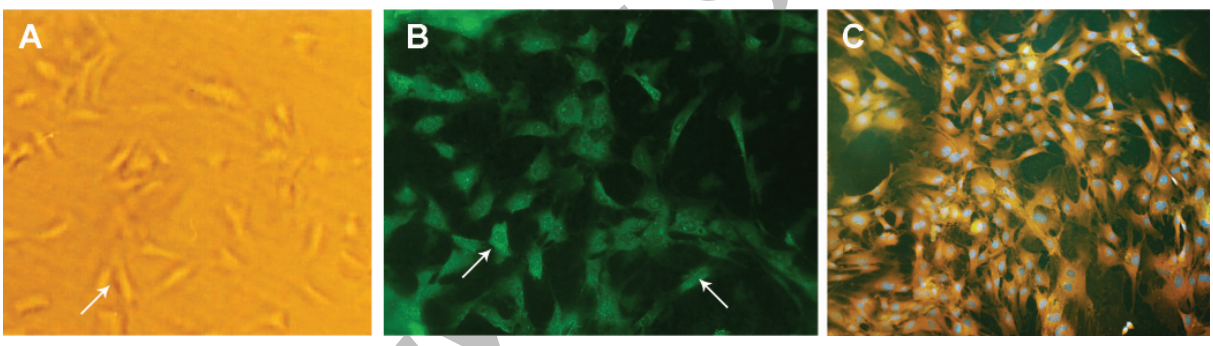

Fig 4: A) Cultured Schwann cells in P3 stage. For Schwann cell purification, the culture was incubated in ara-C to remove fibroblasts from the Schwann cell culture. B) Schwann cells stained with S-100. The fusiform morphology of these cells and their nuclei can be easily observed. C) Schwann cells stained with Dil and DAPI .The nuclei and cytoplasm of Schwann cell are stained with blue and dark yellow colors respectively.

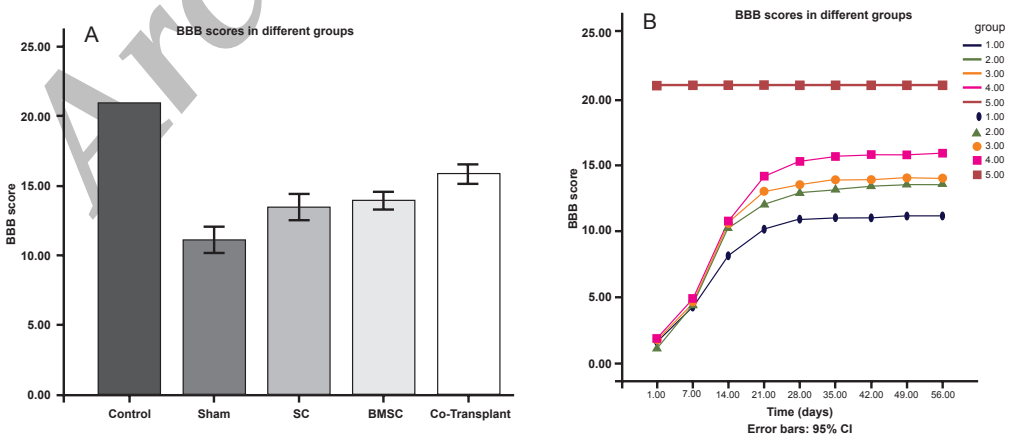

Fig 5: Line graph representing the assessment of motor function recovery by BBB scores following the contusion injury. During the first 2 weeks after injury, functional recovery rates in all groups were similar, but from 3-8 post injury weeks, animals in the experimental groups exhibited significant BBB score increases compared to the sham groups .There were significant differences between the co-transplant and the SC & BMSC groups ($p<0.05$), but there was no significant difference between the SC and the BMSC groups ($p>0.05$). Values represent Means ± SD.

The BMSCs were cultured and labeled with BrdU. the immunohistochemistry process that followed showed that after 8 weeks, BrdU-positive BMSCs survived and gathered around the cavity center (Fig 3). The SCs were harvested and labeled with Dil and DAPI. The fluorescence microscopy that followed showed DiI-/DAPI labeled Schwann cells and confirmed that these cells had an exogenous source (Fig 4C).

Immunohistochemistry for S-100 was carried out and the results after 8 weeks showed that S-100 positive Schwann cells could survive for a long time and exert their neuroprotective effects improving locomotor recovery and axonal regeneration (Fig 4B).

The effects of BMSCs on functional recovery and promotion of axonal regeneration have been studied in various models of spinal cord injury in rats (29, 30). For instance, Tiraihi et al. have reported that intraspinal and intravenous transplantation of BMSCs can significantly improve functional recovery in rats (8, 9). Their data have shown that both intravenous and intraspinal transplantation of BMSCs can result in promotion of behavioral recovery. Researchers have explained several mechanisms which may cause the promotion of functional improvement by BMSCs. Some believe that BMSCs secret many factors such as: neurotrophic factors which induce tissue plasticity (31), neuroprotective factors (33) and brain natriuretic peptides which reduce neuronal apoptosis (34).

Other researchers explain that BMSCs are multipotent cells capable of differentiating into neurons and glial cells in specific in vitro (36) and *in vivo* (37) conditions; hence, these neurons and glial cells can replace damaged cells (35), regenerate injured spinal tissue and promote behavioral recovery.

In another study, Ohta et al. demonstrated that intraventricularly injected BMCs do not differentiate into neurons and glial cells, but they promote functional improvement (38). They suggested that transplanted BMSCs produce trophic factors in the cerebrospinal fluid (CSF) which are beneficial for neurons and glial cells, (39,40) and this induces behavioral improvements.

It is reported that BMSCs can secret nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), human growth factor (HGF) and vascular endothelial growth factor (VEGF) (41) and induce glial cell production of neurotrophic factors like NGF and BDNF (42).

Hofstetter et al. (32) reported that injected BMSCs one week after SCI formed bundles bridging the epicenter of the lesion. These bundles are formed by immature astrocytes formed from stem cells in response to the injury.

One of effective cell candidates used in cell transplantation after SCI is the Schwann cell (SC). This cell is well-known for its promotion of repair in the injured spinal cord.

SCs secret various neurotrophic factors which can improve the microenvironment at the site of injury and induce improved locomotor recovery in injured rats. These factors include the brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF). SCs can also produce some extracellular matrix molecules such as laminin to which injured axons can attach (43, 44).

SCs have an outstanding ability to protect their survival in the injured tissue. Some autocrin factors enabling their long-term survival in the injured area include laminin, neurotrophin-3 (NT-3), insulin-like growth factor (IGF) and platelet-derived growth factor (PDGF) (46).

Investigators believe that SCs can myelinate demyelinated axons and guide regenerating axons into central tracts (45).

Although the efficacy of individual transplantation of BMSCs and SCs have been proven, the number of regenerated axons is small and functional improvement is modest; therefore, we decided to investigate a possible improvement of results from co-transplantation of these cells.

The BBB scores of the BMSC and SC groups in our study 8 weeks after injury are nearly similar to scores in other studies. For instance, the scores of BMSC injected animals in the Hofstetter et al. (32) and Ohta et al. (38) studies are 13 and 13.87 ± 3.0 respectively. The BBB scores of SC injected animals in a Ban et al. (47) and a Firouzi et al. (48) study were 13.17 ± 0.71 and 13.5 ± 1.1 respectively and are similar to our scores.

Our data indicate that combinational injection of BMSCs and SCs in the injured rat spinal cord improves locomotor recovery more considerably than individual injections of BMSCs or SCs.

In the co-transplant group, the numbers of injected BMSCs and SCs were equal. In other words, the ratio of BMSCs to SCs received by animals in the co-transplant group was 1:1.

It is difficult to determine which cell type is more effective in the improvement of BBB scores, but it is proposed that BMSCs, SCs, their secreting factors along with other factors work in concer.

It is proposed that post SCI injected BMSCs express neurotrophic and growth factors which prevent neuronal apoptosis and result in neuronal and glial cell survival leading to improved behavioral

recovery. Furthermore, these factors provide a suitable environment that promotes axonal growth resulting in locomotor recovery. Moreover, BMSCs can improve vascularization through the expression of VEGF leading to damaged neural tissue repair. BMSCs probably participate in partial behavioral recovery thru these mechanisms.

SCs can form a cellular bridge crossing the lesion site and provide a physical support for axonal growth; they also produce neurotrophic factors and cell adhesion molecules leading to axonal growth promotion. The main role of these cells is myelin formation production in demyelinated axons. It is proposed that all of these mechanisms are probably responsible for improvements in partial locomotor recovery. It is supposed that BMSCs, SCs and their secreting factors act as synergists and result in the axonal repair which leads to improved BBB scores and locomotor recovery.

BMSCs and SCs have some advantages in comparison to other cells commonly used in cell therapy. They can be easily isolated and cultured, and their transplantation is safe and without immunological reactions; therefore, they can be employed in autograft transplantation therapy in patients with spinal cord injury.

Our findings suggest that combinational administration of BMSCs and SCs one week after SCI can improve locomotor recovery in rats and it may be employed as a useful method for treatment of human spinal cord injury without surgical intervention; hence, further studies are required for the development of this method as well as future treatment of human SCI.

In summary, the data in this study show that co-transplantation of BMSCs and SCs in an animal model of SCI can more considerably enhance locomotor recovery than transplantation of these cells individually and may be a beneficial treatment method for SCI.

Conclusion

This study shows that co-transplantation of BMSCs and SCs may provide a powerful therapy for SCI and is required for the development of future combinatory treatment strategies.

Acknowledgement

This study was financially supported by a grant from the Cellular and Molecular Research Center at Iran University of Medical Sciences. The cryosectioning process of this research was performed at the anatomy department of Tarbiat Modarres University. We express our deep appreciation for Dr. Salehnia, Mrs. Ebrahimi and Mr. Pourbeyramvand's support. There is no conflict of interest in this article.

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