BENEFICIAL EFFECTS OF BONE MARROW DERIVED MESENCHYMAL STEM CELLS (BM-MSC) IN FUNCTIONAL RESTORATION OF CHRONIC SPINAL CORD INJURY EXPERIMENTALLY INDUCED IN MALE ALBINO RATS: COMPARISON OF INTRAVENOUS AND INTRALESIONAL ROUTS OF ADMINISTRATION

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ABSTRACT

Background: The failure of regeneration after spinal cord injury (SCI) has been attributed to axonal demyelination and neuronal death. The main goal of basic SCI research is to develop new therapeutic interventions which can be applied to prevent or reduce disability.

Objectives: The aim of the present study was to investigate the effects of rat bone marrow-derived mesenchymal stem cell (BM-MSC) transplantation on the functional restoration of spinal cord injury (SCI) in rats after systemic and intralesional injection.

Material and methods: 40 adult male albino rats were utilized with an average weight 150-200 grams for each. Animals were divided into four groups: control group, positive control group and two experimental groups for intravenous and intralesional injections. The samples were taken from spinal cord around the region of spinal cord injury to study the processes of nerve damage and repair by using Histological study (H&E) staining, immunohistochemical study for detection of nerve regeneration and astrocyte response to the injury.

Results: There were obvious differences in the results of the BBB score, spinal cord immunostaining results after intravenous and intralesional injection in favor of the intralesional route.

Conclusions: Bone marrow-derived mesenchymal stem cell transplantation has a promising role in functional restoration of the spinal cord in rats after chronic spinal cord injury especially with the intralesional route of injection.

Keywords: spinal cord injury, bone marrow-derived stem cells

Spinal cord injury (SCI) results in neuronal degeneration and demyelination due to oligodendrocyte apoptosis at the region of trauma resulting in severe functional impairment of motor and sensory pathways.39

Much of the morbidity associated with SCI occurs due to the limited intrinsic ability of the spinal cord to recover following transection or contusion. The pathophysiology of SCI is considered biphasic in nature. Primary injury results from mechanical force injuring the spinal cord, tearing axons, blood vessels, and causing cell membrane disruption. Secondary injury occurs via the subsequent edema, ischemia, inflammation, cytokine production, free radical damage, glial scar formation, apoptosis and necrosis that ensue. The failure of regeneration after spinal cord injury (SCI) has been attributed to axonal demyelination and neuronal death.3

The main goal of basic SCI research is to develop new therapeutic interventions which can be applied to prevent or reduce disability. Mesenchymal stem cell (MSCs) lineage is a kind of self-renewing and multipotent stem cell, which was initially identified from the bone marrow.7,8

The aim of the present study was to investigate the effects of rat bone marrow-derived mesenchymal stem cell (BM-MSC) transplantation on the functional restoration of rats' spinal cord in chronic SCI both with the intravenous and the intralesional routes.

MATERIAL AND METHODS:

Animals: 40 adult male albino rats were utilized with an average weight 150-200 grams for each. The animals were housed in special cages and were maintained on standard
laboratory conditions, were fed with adequate available tap water and commercial diet. All ethical protocols for animal treatment were followed and supervised by the animal facilities.

**Preparation of BM-derived MSCs from rats:**

**1-Isolation of BM-derived MSCs from rats:**

Bone marrow was harvested by flushing the tibia and femur of rats with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Nucleated cells were isolated with density gradient and resuspended in complete culture medium supplemented with 1% penicillin-streptomycin. Cells were incubated at 37°C in 5% humidified CO2 for 12-14 days. Media was changed every 2-3 days. When large colonies developed (80-90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and cells were trypsinized with 0.25% trypsin in 1 ml Ethyline Diamine Tetra Acetate (EDTA) (GIBCO/BRL) for 5 minutes at 37°C. After centrifugation, cell pellet were resuspended with serum-supplemented medium and incubated in 50 cm culture flask. The resulting cultures were referred to as first passage cultures.

**2-Morphological identification of BM-derived MSCs:**

MSCs in culture were adhesive and fusiform shape. The bone marrow derived MSCs were labeled by PKH26 (red fluorescent cell linker), examine the cells using fluorescence microscopy. The stained sample should be checked for cell recovery, cell viability, and fluorescence intensity. The appearance of labeled cells may vary from bright and uniform labeling to a punctate or patchy appearance.

**Spinal cord injury:**

Aseptic T9–T10 laminectomy under anesthesia (pentobarbital sodium; dose 50–80 mg/kg IP; Hospira, Lake Forest, IL) was performed. Spinal cord contusion was induced using a weight-drop apparatus, where a guided 10 g rod was dropped 12.5 or25 mm onto the exposed dura mater, representing moderate or severe SCI, respectively. Following contusion, the incision was closed with wound clips. The rats received 0.02% amoxicillin in the drinking water to prevent infection throughout the duration of the experiment. Body temperature of 37°C during anesthesia was maintained by a temperature-controlled heating pad and recorded with a rectal thermometer. During the recovery, urine was expressed manually twice daily to assist in urination until intrinsic function returned to normal.

**Animal grouping and treatment:**

Animals were divided into four groups. Each animal underwent good feeding, hygiene and hosing.

- **Group I (negative control group):** Formed of 10 rats and underwent no spinal cord operation with daily bladder evacuation and good hygiene.
- **Group II (positive control group):** Formed of 10 rats and underwent spinal cord operation causing paraplegia with daily intramuscular penicillin, bladder evacuation and good hygiene.
- **Group III (experimental groups):** Formed of 20 rats and divided into two subgroups each containing 10 rats:
  - **Subgroup (a):** Each animal was undergo spinal cord injury operation causing paraplegia with daily intramuscular penicillin administration, bladder evacuation and good hygiene, then single intravenous dose of BM-derived MSCs at 8th week.
  - **Subgroup (b):** Each animal was undergo spinal cord injury operation causing paraplegia with daily intramuscular penicillin administration,
bladder evacuation and good hygiene, and single intralesional dose of BM-derived MSCs at 8th week. The samples was taken from spinal cord around the region of spinal cord injury to study the processes of nerve damage and repair by using Histological study (H&E) staining, immunohistochemical study for detection of nerve regeneration and astrocyte response to the injury.

**Histomorphometry:**
Histomorphometry was performed using an imaging system constituted by a digital Q-color 5 cameras (Olympus, Tokyo,) coupled to an epifluorescence Axiovert 100 microscope (Carl Zeiss, Thornwood, NY). Randomly picked fields of sections were captured from each animal, using a magnification 4 objective lens. Quantification was estimated by the percentage of stained area in comparison to the total area of fields examined, using Image-Pro Plus 5.0 (Media Cybernetics, Bethesda, MD) image analysis software.

**Immunostaining:**
- Using the ultravision detection system. Anti-polyvalent, HRP/ DAB (ready to use)
- **IHC staining technique:** The reagent in this kit constitute a labeled streptavidin-biotin immunoenzyemantic antigen detection system. This technique involves the sequential incubation of specimen with an unconjugated primary antibody specific to the target antigen, a biotinylated secondary antibody which reacts with the primary antibody, enzyme- labeled streptavidin, and substrate chromagen. This system is an extremely sensitive method.

**Reagent provided:**
- **Vial 1:** peroxidase blocking reagent: 0.3% hydrogen peroxide containing sodium azide.
- **Vial 2:** ultra v block conjugated to goat anti-HCL buffer containing carrier protein and an antimicrobial agent.
- **Vial 3:** Biotinylated goat antibody as a link antibody.
- **Vial 4:** Streptavidin peroxidase.
- **Vial 5a:** Buffered substrate; buffer substrate solution, PH7.5, containing hydrogen peroxide and a preservative.
- **Vial 5b:** DAB+ chromagen; 3, 3, -diamino-benzidinechromagen solution (for each 1 ml buffer vial 5a, one drop from vial 5b (DAB + chromagen) was added to be ready to use.

**Preparation of control slides:**
Each staining run included both negative and positive control slides to confirm that the staining system is working properly.

- **Positive control:** Sections of rat liver known positive reaction to MMPS and TIMPS were used as positive control.
- **Negative control:** These slides were prepared from the same tissue block and instead of using the primary antibody; the normal/non immune rat serum was used.

**Evaluation of motor and sensory function**

**Open-field locomotion**
The rats were placed in an enclosure and scored by two blinded observers according to the Basso, Beattie, and Bresnahan (BBB) rating scale before contusion, 2–3 days after contusion, and once a week after transplantation for 8 weeks. Briefly, all rats were individually tested for 5 min on the floor and then in the cage to obtain the overall score (maximum 21 points). Inter-rater reliability was 95%.
The BBB scale has a range from zero (no hind limb movements) to 21 (normal coordinate gait), using paw placement, joint movement, and truncal stability as important factors in determining the level of functional recovery. Scores in the 0 to 7 range focus primarily on hip, knee, and ankle joint movement, the 8 to 13 range keys in on paw placement and coordination, and scores of 14 to 21 rely heavily on trunk stability, tail position, and paw placement.

**Electrophysiological Assessments**

Prior to the start of the experiments, electrode pedestals were implanted into the skull of the rats for electrophysiological assessments. Briefly, a standard dental drill was used to drill four burr holes into cranium at locations of the primary somatosensory cortex corresponding to the hind limbs (2.8 mm lateral, 2.5 mm posterior to bregma) and forelimbs (3.8 mm lateral, 0.2 mm posterior to bregma). A fifth electrode on the right frontal bone was inserted as the intracranial reference. Transcranial screw electrodes (E363/20, Plastics One Inc., Roanoke, VA) were then screwed into the holes such that they made very light contact with the dura mater, and they were mounted to an electrode pedestal (MS363, Plastics One Inc., Roanoke, VA) using dental cement.

For SSEP recordings, the rats were anesthetized with 1.5% isoflurane. Intramuscular needle electrodes (Safelead F-E3-48, Grass Technologies, West Warwick, RI) were used to electrically stimulate the median and tibial nerves of the forelimbs and hind limbs, respectively. For each session, each of the 4 limbs was stimulated in an alternating fashion and the corresponding SSEPs were recorded. Stimuli were provided at 1 Hz such that each limb received a pulse at a frequency of 0.25 Hz (3 mA, 200 ms pulse width). A reference ground electrode was placed subdermally at the back of the neck. Signals were amplified with a gain of 20,000 and sampled at 4882 Hz using a custom designed TDT System (Tucker-Davis Technologies, Alachua, FL).

For each recording session, at least 200 sweeps were recorded. Two pre-injury baseline recordings were taken prior to injury for each rat. Following injury, SSEPs were recorded in twenty-minute sessions preinjury, postinjury and immediately pretreatment and eight weeks posttreatment. Signal processing was performed using MATLAB 7.0 (MathWorks Inc., Natick, MA). To improve signal-to-noise ratio, each sweep was high-pass filtered (20 Hz cutoff), notch filtered (50–70 Hz), and mean corrected. The mean of the first 200 sweeps of each recording session was taken for further analysis, for which the peak-to-peak amplitude and latency to the first positive peak were identified.

**Evaluation of bladder function (Awake cystometry)**

Eight weeks after transplantation, the rats were anesthetized using isoflurane, and a catheter was implanted in the bladder. Briefly, the bladder was exposed by a midline lower abdominal incision, and a polyethylene catheter (PE-60; Clay Adams, Parsippany, NJ) was implanted into the bladder through the dome. The bladder catheter was tunneled subcutaneously and exited through the skin on the back, as previously described. The rats were then placed in a restraining cage (KN-326; Natsume, Tokyo, Japan) and allowed to recover for 1–2 h. The bladder catheter was connected to a pressure transducer (World Precision Instruments), and a microinjection pump (STC-523; Terumo, Tokyo, Japan). Room-temperature saline was infused at a rate of 0.1 mL/min, and intravesical pressure was recorded to compare urodynamic parameters in each group. The maximal voiding pressure, post-void residual urine (obtained by emptying the bladder at the end of the
last recorded micturition using a syringe attached to the catheter that was inserted in the bladder), bladder capacity (urine volume/void + residual urine), and the frequency of detrusor hyperreflexia (DHR) per micturition episode (peaks above 2 SD of the baseline recording) were compared in each group.

**Statistical analysis:**
A two-way analysis of variance (ANOVA) comparing control versus experimental groups over time, with time taken as a repeated measure, was used to analyze the data, with significance set at \( p < 0.05 \). Post-hoc analysis was performed using the Bonferroni test. One-way ANOVA was used for changes in the spinal cord.

**RESULTS:**

**Motor and sensory function:**
Table 1: Open-field locomotion (BBB SCORE):

**Electrophysiological Assessments:**

Table 2: SSEP results

Following SCI, the SSEPs of both the injury and transplantation groups were significantly reduced in amplitude with increased latencies. These two parameters represent the amount of signal transduction through site of injury and the speed at which the signal travels, respectively. As a number of demyelinated but intact axons still exist following SCI, enhancements in SSEPs following cell therapy would reveal a remyelination of spared axonal pathways that result in increased conductivity. By the eighth week post-transplant, the amplitude of SSEPs for the treatment group was significantly greater than the injury-only group (58.6±10% versus 33.6±5%, respectively; \( P=0.009 \))

The latency was also evaluated. This is the time from stimulation at the periphery to the appearance of the first positive peak of the SSEP waveform, and as such can be used as an indicator of the speed of conduction of sensory pathways. The treatment group showed a significant improvement, resulting in a latency by week 8 that was similar to the baseline prior to injury (102.6±6%).

**Bladder function (Awake cystometry)**

Table 3: Awake cystometry (mean± standard deviation)

Cystometric data were collected at 8 weeks post-transplantation, prior to sacrifice. Voiding volumes were significantly higher in all contused animals compared to non-injured animals. No significant differences were observed between the injured and the transplantation groups regarding the maximal detrusor pressure. Bladder capacity of the transplantation rats was significantly higher than that of animals in the other groups. No difference in post-void residual urine was observed in any of the animals.

**Changes in the spinal cord**

Table 4: Histomorphometry:

**Histological finding**

H&E staining:
Control group 1: revealed normal. View of the grey matter of the spinal cord which is clearly surrounded by white matter. The ventral horn has a number of processes which
extend into the white matter. The tissue displays a number of multipolar cells that are relatively large compared to the nerve fibres which surround these cells. Although at this magnification it is not possible to definitively identify these cells but it is likely that they are cell bodies of neurons. The surrounding nerve fibres are likely to be neuroglial cells, although the cytoplasm of these cells is not visible. The seemingly poor organisation of the tissues surrounding these neurons is often referred to as neuropil (fig 1). Meanwhile Untreated group II: spinal cord tissue sections 8\textsuperscript{th} week post operation showed hemorrhagic focus in the grey and white matters with complete destruction of the tissue, this was represented in neuron dissolution giving liquefied appearance in grey matter (Fig.2,3). Sections of this injured group showed as well eosinophilic (degenerating) neurons with darkly stained (pyknotic) nuclei. Swollen cells and many vacuoles were demonstrated. Some dark irregular shrunken neurons were detected. There was apparent cellular destruction and necrosis as compared to all other groups. Group III in which animals were intralesional treated with BMSCs spinal cord tissue showed: apparently more glia cells and less dark neurons as compared to both normal control and untreated groups (Fig.4). In addition, some areas showed inflammatory infiltrates, pyknotic cells surrounded by diffused glial cells & with mild vacuolation. 

Group IV in which animals were treated intra venous with BMSCs H&E spinal cord tissue sections showed normal structure of spinal cord tissue: the neurons are clearly visible, the outer limits of the grey & white matters are marked and there were no cystic spaces (Fig5). However, a small amount of hemorrhaging was observed in the spinal cord of a few rats and there was a slight gathering of gliocytes. This may have been associated with a reduction in the stability of the spine and the subsequent injury of the spinal cord for the resection of the vertebral plate.

\textbf{GFAP immunohistochemical results}

Anti-GFAP stained spinal cord sections: spinal cord transverse sections of control group revealed brown immunostaining in the cytoplasm and processes of astrocytes in all regions in white and grey matter. The immunoreactive cells were detected mainly in a regular form in both regions. Untreated group revealed apparently strong immunoreactivity as compared to control and all other groups; the immunoreactive astrocytes were detected mainly in all spinal tissues closely related to site of the lesion. The dense packing of intensely GFAP-positive astroglial processes between the spaces made it extremely difficult to comment on changes in astrocytic cell body density (fig 6). Comparing both Intravenous and intralesional treated groups to the untreated group Anti --GFAP stained spinal cord sections showed moderate reactivity which was restricted to the place of lesion as going further away from the lesion site, no significant astrocytic reaction could be detected.

In the GFAP immunohistochemical staining, the cytoplasm of the positive cells was brown and radially formed, spider-like projections were observed. At the 8\textsuperscript{th} week post-SCI, the staining in the control group FIG.(6) showed a small volume of positive cells, with a relatively sparse density. The nerve structure was visible. At the 8\textsuperscript{th} week post-SCI, the number of GFAP-positive cells in group II was increased. As shown in Fig. 7, the cells were deeply stained, showing hypertrophy and neurite extension. A number of positive cells surrounded the cystic cavity. Astrocyte proliferation and hypertrophy were also observed near the injury, although the extent of the proliferation was less than the injury area. At 8\textsuperscript{th} week post-SCI, the number of GFAP-positive cells in group III FIG (8) had significantly reduced compared with that of group II post-SCI. However, the prominence of the positive cells was thicker and longer, woven into reticulate structure and formed a dense glial scar. There were more
GFAP-positive cells in group II than in the MSC treated group. As shown in Figs. 9, at the 8th week post-SCI, the number of GFAP-positive cells in group IV was significantly reduced and the GFAP-positive cells became smaller, with a reduced prominence and pale staining. In addition, the scope of the glial scar was smaller.

**DISCUSSION:**

Cell transplantation may promote neural regeneration and rescue impaired neural function after spinal cord injury by means of (1) secreting permissive neurotrophic molecules at the lesion site to enhance the regenerative capacity; (2) providing a scaffold for the regeneration of axons; (3) replacing lost neurons and neural cells. An early stem cell transplantation study in humans was reported as a one patient case report by a Korean research team in 2005. Multipotent adult stem cells from umbilical cord blood were directly injected into the lesion site of a SCI patient who had been nonstanding disabled for years, and the ability to walk was reported to be restored. Within the field of regeneration research after CNS injury, MSCs are being advocated as a promising cell source for repair because of the following excellent properties. First, the acceptance from the donor and the isolation from cryopreservation are relatively easy and simple. Second, the expansion of cells to clinical scales can be achieved in a relatively short period of time. Third, the preservation of MSCs with minimal loss of potency can be performed conveniently. Fourth, transplanted MSCs are capable of decreasing demyelination, reducing neural inhibitory molecules, of promoting axonal regeneration, and of guiding axon growth. Lastly and importantly, there are no reports of adverse reactions to allogeneic versus autologous transplants, and allogeneic MSCs are well tolerated and do not elicit immediate or delayed hypersensitivity reactions.

Transplantation of MSCs in SCI animal models has been applied by several groups to promote sensorimotor function recovery and bladder function recovery via neural lineage differentiation, neurotrophic paracrine effects and posttraumatic inflammation regulation. As Nakajima et al. reported, the activation of macrophages in the post-SCI inflammatory environment can be regulated by the transplantation of MSCs. After transplantation into the contusion epicenter, the undifferentiated MSCs significantly upregulated the level of IL-4 and IL-13, and downregulated the level of TNF-alpha and IL-6. These changes of inflammation factors resulted in the shielding of macrophage phenotype from M1 (iNOS- or CD16/32-positive) to M2 (arginase-1- or CD206-positive). With the alteration of macrophage phenotype, more preserved axons, less scar tissue formation and increased myelin sparing were observed, furthermore, locomotion recovery in the MSCs transplantation group was confirmed. In another MSCs transplantation trial, Karaoz et al. claimed significant motor recovery in the MSCs implanted group, however, only Nestin+/GFAP+ astrocytic-like cells were observed at 4 weeks after transplantation. By implanting human MSCs into the contusion rat model, more rapid restoration of hind limb function was achieved when compared with other control groups, but significant differences of BBB scores and coupling scores among all groups were not obtained. More importantly, bladder function was not restored in either group. In addition to motor function deficits and bladder dysfunction, neuropathic pain is also a common and debilitating symptom in SCI patients which is induced by abnormal neuronal activities in the spared tissue surrounding the lesion site. In order to clarify the relationship between chronic inflammation and the therapeutic effects of MSCs on sensory deficits, Abrams et al. evaluated chronic inflammation, posttraumatic cyst formation, and mechanical and thermal sensation thresholds of contusion SCI rats treated with MSCs transplantation. After MSC injection at three different sites (the
lesion site, rostral and caudal to the lesion), the injury-induced sensitivity to mechanical stimuli was significantly attenuated, although no effect was observed on injury-induced sensitivity to cold stimuli. More importantly, GFAP + reactive astrocytes and ED1+ macrophages/microglia, assessed as a measure of the chronic inflammatory response, were significantly attenuated by MSCs administration. The improvement of locomotor function in SCI rats by means of MSCs transplantation was also reported.

However, the therapeutic in vivo application of MSCs for spinal cord injury might face a series of challenges which include low survival rate of grafted cells (5–10%), the lack of neural differentiation, glial scar formation, cystic cavity formation, the inhibitory cellular environment, the transplantation time point, and the graft/host immune responses.10 In addition, different transplantation routes can also bring different outcomes after MSCs transplantation. In this series there were an obvious difference in the results of the BBB score after intravenous and intralesional injection in favor of the intralesional route. In a comparison experiment, Kang et al. compared the BBB motor scores of SCI rats between intravenously (IV) and intralesionally (IL) transplanted groups.16 The fates of engrafted allogenic MSCs in two different groups were also investigated. Based on their results, the NeuN positive neural differentiation and CC-1 positive oligodendroglial differentiation of engrafted MSCs was observed in the IL group, and GFAP positive astrocyte differentiation was observed in the IV group. Meanwhile, the expression of both BDNF and NGF in the IL group was significantly higher than the IV group. This phenomenon was suggested to be related to the absolute number of the engrafted MSCs. Regarding motor function recovery, both MSC transplantation groups achieved significantly better outcomes than the control group (BBB scale 6.5 ± 1.8). The BBB scores in the IV group (11.1 ± 2.1) were significantly better than the IL group (8.5 ± 2.8). The authors suggested that the non-favorable motor function improvement in IL group might be related to the additional injury during the transplantation in the intralesional injections.

By means of intravenous transplantation of Lac Z reporter gene transduced MSCs in the earlier postinjury infusion time, Osaka et al. reported significantly improved locomotor recovery in severe contusive SCI rats, and they suggested that the minimal invasive, intravenous cell administration is a prospective therapeutic approach in acute and subacute SCI.29 Mothe et al. investigated the effects of another transplantation approach, intrathecal transplantation, with neural stem/progenitor cells (NS/PCs) and bone-marrow derived mesenchymal stromal cells (BMSCs).26 Most of transplanted cells were showed to remain in the intrathecal space, and neither NS/PCs nor BMSCs migrated into the parenchyma of the injury site.

After implantation into the injured spinal cord, the neuronal differentiation of MSCs in vivo is not efficient and the lack of neuronal markers expression has been reported in some transplantation studies.10 Without neuronal differentiation, the engrafted MSCs may generate a favorable environment for functional recovery through modulating the post-SCI inflammatory response and by having neurotrophic paracrine activity.12 As Boido et al. reported, significantly reduced lesion volume and improved hind limb sensorimotor functions were observed after mouse MSCs were transplanted into the lesion cavity of compression SCI mouse model, even though the engrafted MSCs were observed to be neuronally undifferentiated and astroglial and microglial activation was not altered.5 Gu et al. also reported similar results, the reduced volume of post-SCI cavity and increased spared white matter were observed after
transplantation of bone marrow mesenchymal stem cells into the epicenter of the injured spinal cord of rats. Interestingly, despite the lack of expression of neuron, astrocyte, and oligodendrocyte cell markers, an increase in the number of axons in MSCs transplanted rats was confirmed via transmission electron microscopic examination. In the in vitro experiment of the same study, Gu et al. investigated the paracrine activity of MSCs by means of a MSCs and spinal neuron coculture system. Their results confirmed the expression of brain-derived neurotrophic factor (BDNF) and glia cell line-derived neurotrophic factor (GDNF).

The therapeutic effects of MSC transplantation on the sensorimotor deficits in animal SCI models have been clearly confirmed by a large number of studies. In order to overcome the potential problems associated with direct transplantation of undifferentiated MSCs, researchers have tested several modifications of transplantation strategies, such as pretransplantation neural differentiation, neurotrophic gene transduction, glial cell cotransplantation, and tissue engineering. The neural pretransplantation differentiation is the most commonly used strategy to promote the therapeutic effects of engrafted MSCs. Rodent MSCs are able to efficiently differentiate into neural precursors by culturing with basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and heparin. One method of human MSC neural differentiation was described by Alexanian et al. in 2011.

According to his method, human MSCs were exposed to histone deacetylases inhibitor (Trichostatin), DNA methyltransferase inhibitor (RG-108), biologically active form of cAMP, and phosphodiesterases inhibitor (Rolipram) in a medium consisting of NeuroCult/N2 supplemented with bFGF for two weeks before transplantation. Park et al. reported a new method to generate functional motor neuron (MN)-like cells from genetically engineered human MSCs. They transduced motor neuron-associated transcription factor gene expression into the human MSC, then they treated the genetically engineered MSCs expressing Olig2 and Hb9 with optimal MN induction medium. By using an ex vivo model of SCI, they showed that these reprogrammed MSCs exhibited characteristics of MN-like lineage and are potentially therapeutic for autologous cell replacements.

Alexanian et al. injected neural modified bone-marrow derived MSCs rostral and caudal to the T-8 lesion immediately after injury. 12 weeks after SCI, locomotor function was significantly improved by the neurally modified MSCs, and the volume of lesion cavity and white matter loss were significantly reduced. However, the improvement of thermal sensitivity was not observed. Cho et al. transplanted neurally differentiated rat MSCs (NMSCs) into the epicenter of a contusive lesion, thereafter, the BBB scores, somatosensory evoked potentials (SSEPs) and motor evoked potentials (MEPs) were evaluated. Nine weeks after NMSCs transplantation, the recovery of motor function was reported, and significantly shortened initial latency, N1 latency and P1 latency of the SSEPs were observed. Pedram et al. utilized a Fogarty embolectomy catheter to create a contusion lesion at T8-9 level of rats’ spinal cord, then the autologous neural differentiated and undifferentiated MSCs were cotransplanted into the center of lesion cavity. Five weeks after transplantation, the BBB scores in both cotransplantation group and predifferentiation group were reported to be significantly higher, when compared with undifferentiated group, respectively. However, no significant difference between cotransplantation and predifferentiation groups was observed.

In addition to neural predifferentiation, neurotrophic gene transfection has also been tested in some MSC in vivo studies. Liu et al. implanted bFGF transgene expressing...
rat MSCs into the SCI rat model and reported a significantly higher BBB score in the bFGF group when compared with control groups at 3 weeks after the injection. Furthermore, significantly more bFGF-positive neurons were observed in the bFGF group, and significantly higher optical density values of NF200-positive neurons and MBP-positive axons were also demonstrated in the bFGF group. Therefore, they suggested that the bFGF gene-modified MSCs might be effective in promoting axon regeneration and functional recovery after SCI. In another in vivo study using gene modified MSCs, Zhang et al. investigated the therapeutic effects of Neurotrophin-3 (NT-3) gene modified MSCs in anethidium bromide (EB)-induced demyelination SCI model of rats. 21 days after the administration of NT-3 modified MSCs, locomotor function was improved, and similar to that in the saline injured control group. The improvement was significantly better than the other groups which include MSC group, Lac Z gene modified group, and EB injured group. Similar improvements of spinal cord evoked potentials (SCEP) amplitude and SCEP latency were also achieved in the NT-3 modified MSCs group. Via immunostaining, significantly higher number of NG2- and APC-positive engrafted MSCs were observed in the demyelination site of the spinal cord after transplantation of NT-3 modified MSCs at the end of experiment.

In order to provide a favorable environment for neural regeneration and to support the survival of implanted cells and their neural differentiation, the use of biologic scaffolds has drawn increasing interest. Zurita et al. developed a biologic- scaffold system from blood plasma, called platelet-rich plasma (PRP) scaffolds. According to their report, most of the cocultured human MSCs demonstrated optimized capabilities of survival and neural differentiation after the administration of BDNF. In 2011, a gelatin sponge (GS) scaffold system, which was constructed by ensheathing GS with a thin lm of poly-(lactide-co-glycolide) (PLGA), was reported by Zeng et al. Based on their work, this GS scaffolds system was able to provide a favorable environment for seeded rat MSCs to adhere, to survive, and also to proliferate. After they transplanted GS scaffolds seeded with rat MSCs into the rat SCI model, a promising result which includes attenuated inflammation, promoted angiogenesis, and reduced cavity formation was reported. In 2012, a combinatorial strategy using a similar PLGA scaffolds system and human MSCs was employed by Kang et al. to evaluate the therapeutic effects on motor function improvements. After PLGA scaffolds seeded with human MSCs were transplanted into a completely transected SCI rat model; significantly higher BBB scores were demonstrated. More importantly, the amplitude of motor-evoked potentials (MEPs) in the combinatorial strategy treated group was significantly higher than the other control groups. In addition, implanted cell survival, neural differentiation, and axon regeneration in the combinatorial strategy group were confirmed by immunohistochemical staining images. In another study, a combination of Matrigel and neural-induced adipose-derived MSCs (NMSCs) was applied by Park et al. to investigate the therapeutic effects on functional recovery from SCI in dogs. 8 weeks after the administration of the combination of Matrigel and NMSCs, a significantly better functional recovery was observed as higher BBB and Tarlov scores. Meanwhile, the reduced fibrosis from secondary injury processes, decreased expression of inflammatory and astrogliosis markers, increased expression of neuronal and neurotrophic markers were also confirmed. Although the bone marrow is the main source of MSCs, scientists have been seeking other sources because bone marrow-derived cells are highly vulnerable to viral infection and the significantly increased cell apoptosis and the loss of differentiation capability that occurs in these cells with age. Alternative sources of MSCs have
been identified by researchers, such as, adipose tissue, amniotic fluid, placenta, umbilical cord blood (UCB), and in several fetal tissues including liver, lung, and spleen. Among all the substitutes for BM-derived MSCs, the UCB is the best choice with many advantages of UCB as compared to BM. The collection of cord blood units is more easier and noninvasive for the donor, the UCB units can be stored in advance and are rapidly available when needed, and the MSCs from UCB is more primitive than the MSCs collected from other sources. Importantly, they are less likely to induce graft-versus-host reactivity due to their immaturity. Ryu et al. investigated the effects of MSCs from different tissues on the regeneration of injured canine spinal cord, which are fat tissue, bone marrow, Wharton’s jelly and umbilical cord blood. Although the differences among four experimental groups were not detected in this study, more neural regeneration and anti-inflammatory activity were observed in the experimental group with umbilical cord blood derived MSCs.

Guo et al. induced human umbilical cord mesenchymal stem cells (hUMSCs) into Schwann-like cells in vitro and grafted these cells into the lesion site of SCI rats. A partial recovery of motor function was reported. Furthermore, neurotrophin-3 (NT-3) administration combined with in vivo transplantation, significantly increased the survival of grafted cells and improved the behavioral test results compared to the cell transplantation only group. Meanwhile, Shang et al. transplanted genetically modified NT-3-hUMSCs to the spinal cord injured rats, and the Basso, Beattie and Bresnahan (BBB) scores and grid tests were applied to evaluate the functional recovery at the end of 12 weeks after SCI. In addition to the promotion of transplanted cell survival, significantly better motor function recovery compared to hUMSCs group was achieved in the NT-3-hUMSCs group. This was associated with intensified 5-HT fiber sprouting, more spared myelin, and reduced cystic cavitation.

The pathological processes at the lesion site in SCI evolve over time, from acute phase, subacute to chronic phase, therefore transplantation at different times postlesion, may have varied effects. The comparison of three different transplantation times (12 hr, 1 week, and 2 weeks after injury) has been explored by Park et al., they injected 1 × 10^6 canine UMSCs into the balloon-induced compression lesion site of experimental dogs in different time groups. The significant improvement of Filby and Tarlov scores, which were used to evaluate functional recovery of the hind limbs, was observed in the 1 week transplantation group, and the accompanying increase in the expression of neuronal markers and decreased expression of inflammation markers were measured as well. In addition, less fibrosis was demonstrated in the 1 week group compared to other groups.

Therefore, it is reasonable to conclude that one week after SCI may be the best time point for the further development of therapeutic studies to obtain neuronal regeneration, reduced fibrosis, and eventual function improvement. In most studies, assessing the long-term effects of treatments is technically difficult due to associated risks of weight loss, urinary infection, and sepsis in injured animals.

However, a 3 year long-term effects study of hUMSC transplantation in dogs with SCI was reported by Lee et al. The hUMSCs were transplanted into the balloon injured lesion site in seven experimental dogs. Despite two transplanted dogs dying within one month after transplantation, four of the five surviving experimental dogs survived for three years. These four dogs had restored the hind-limb motor functions (BBB scores) with significant improvement at three years after injury and deep pain recovery was detected from 5 days post injury. Immunohistochemical staining revealed remyelination with many myelin protein-zero positive axons which is the major structural protein of peripheral myelin.
CONCLUSIONS:
Bone marrow-derived mesenchymal stem cell transplantation has a promising role in functional restoration of the spinal cord in rats after chronic spinal cord injury especially with the intrallesional route of administration.

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