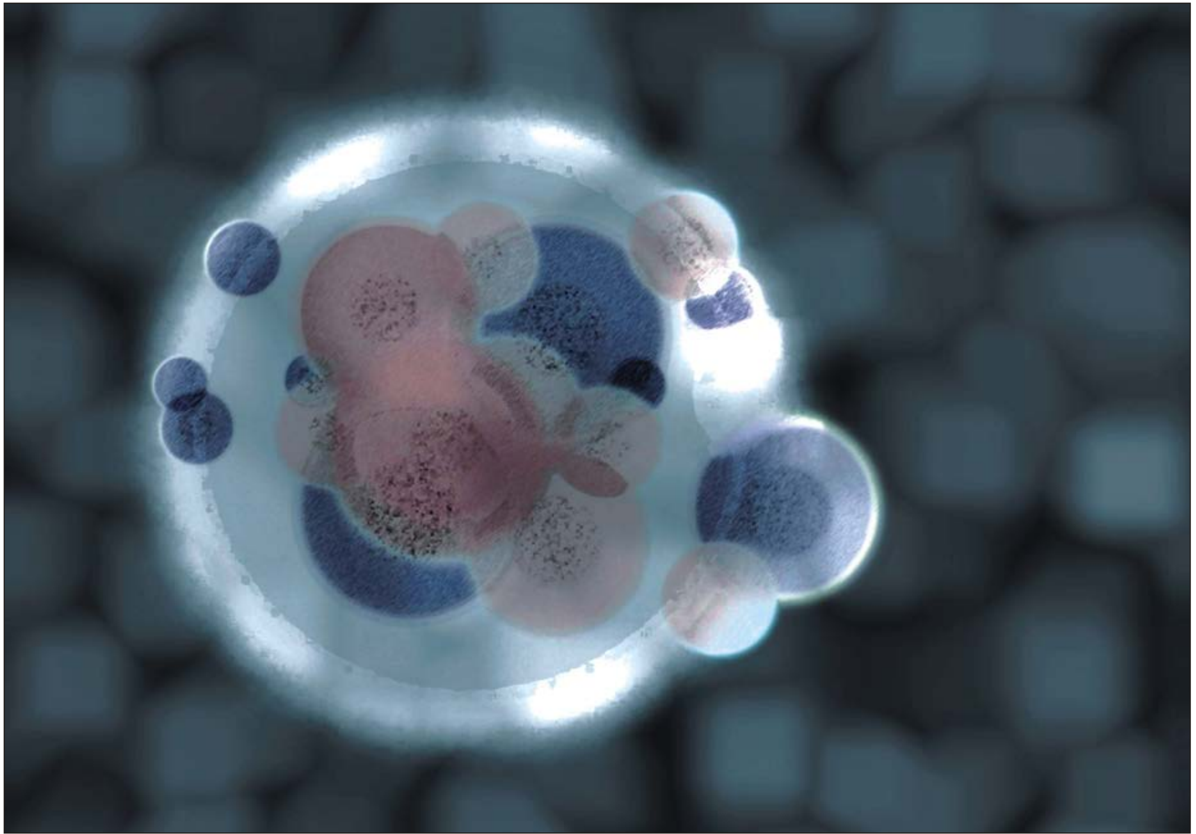

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Bone Marrow Stromal Cells for the Treatment of Spinal Cord Injury in Rats

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Abstract: There are extensive evidences that degeneration process after spinal cord injury could be a good reason for nervous system (peripheral and/or central) problems. In this study the effects of cell administration on Alpha motoneurons regeneration after Spinal Cord Injury (SCI) in rat was investigated. Twenty four male Wistar rats divided to 3 groups (control, Spinal Cord Injury (SCI), (SCI) + stem cell administration n = 8). After 4 weeks the lumber segments of spinal cord were sample, processed, sectioned serially and stained with toluidine blue (pH = 4.65). By using sterological quantitative technique (physical dissector), the number of alpha motoneurons in the right ventral horns of spinal cord were counted and compared with each other. Statistical analysis showed treatment with stem cell at four week significantly ($p<0.05$) reduced neuronal damage and progress the regeneration. The numerical density in this group is near to control group. Then it is consulted that cell administration could reduce the degeneration rate of Alpha motoneurons after Spinal Cord Injury (SCI) in rat.

Key words: Neuronal degeneration, stem cell, numerical density

INTRODUCTION

The discovery of the potential utility of stem cells in neurological repair and regeneration is an exciting development in neuroscience (Nandoe *et al*., 2009). There is limited evidence from the animal model that stem cells improve the functional outcomes in spinal cord injured rats, but the mode by which functional improvement is achieved is for from clear (Coutts and Keirstead, 2008).

In the Central Nervous System (CNS) of adult mammals, spontaneous regeneration of injured neural tissues is very limited (Hayashi *et al*., 2007) since Neural Stem Progenitor Cells ($NSPC_s$) were first shown to exist in the adult mammalian CNS (Hayashi *et al*., 2009). A number of therapeutic strategies to active endogenus ($NSPC_s$) have been developed for the regeneration of injured CNS tissue, including tissue damaged by stroke or spinal cord injury SCI (McDonald *et al*., 2004).

Neural stem/progenitor cells have previously been identified in both the mammalian brain and spinal cord (Horner *et al*., 2000). They have the ability to self-renew and are multipotential for both neurons and glia. Because of these qualities, it has been suggested that they may be useful to repair the spinal cord by generating new cells and an environment that would promote axonal regeneration and several investigators have published studies supportive of this hypothesis (Lu *et al*., 2003).

Transplantation of neural stem cells has been studied extensively for the treatment of central nervous system (CNS) injury (Louro and Pearse, 2008).

It has also been suggested that transplantation of $BMSC_s$ (Dominici *et al*., 2006; Horwitz *et al*., 2005) into the injured spinal cord may provide therapeutic benefit (Chopp *et al*., 2000; Wu *et al*., 2003). Indeed, $BMSC_s$ have been transplanted into several models of central nervous system injury including ischemic stroke, traumatic brain injury and traumatic spinal cord injury. Although there is some evidence for differentiation of $BMSC_s$ into cells of neural linage both *in vitro* and *in vivo*, this is unlikely to be a major factor in functional recovery after brain or spinal cord injury. Other possible mechanisms of recovery after $BMSC_s$ transplantation include neuroprotection, creation of a favorable environment for regeneration, expression of growth factors or cytokines, vascular effects and remyelination. The mechanisms are not mutually exclusive and it is likely that more than one may contribute to functional recovery. The injected cells were conveyed though the CSF and after attaching to the pial surface of the injured spinal cord, migrated and were integrated into the lesion (Bai *et al*., 2003). This indicates that injection of neural stem cells through the CSF is an effective practical way to supply neural stem cells to spinal cord lesions (Bouhy *et al*., 2006).

The aim of this research is study the effect of administration bon stem cells in lateral ventricle of brain after spinal cord injury in rat.

MATERIALS AND METHODS

All experiment was conducted in Faculty of Science, Islamic Azad University of Mashhad, Iran (2009).

Animal subjects: Twenty four male, Wistar rats weighting between 300-350 g served as subjects for these experiments. All animals were housed individually and maintained on a 12/12 light/dark cycle, with lights on at 6.00 h. Ambient temperature in the animal facility was kept at 22±2°C. Food and water was given *ad libitum*.

Preparation of neurospheres: Essentially bone marrow was collected from the femurs of male rats weighting between 300-350 g, resuspended in long-term bone marrow culture medium (approximately $40\text{-}50\times10^7$ cells per rat, seeded at 2×10^6 cells mL^{-1}).The cells were incubated at 37°C in 5% CO_2 (Coutts and Keirstead, 2008). After 3-5 days of culture, the $BMSC_s$ cells formed floating neurospheres which are used for transplantation after two or three passages (Coutts and Keirstead, 2008).

To examine the short-term survival, migration and fate of the $BMSC_s$ *in vivo*, the cells were transplanted at the time of injury and then rats were sacrificed at 28 day after injury.

Surgery for cell transplantation: After inducing anesthesia by intraperitoneal injection of pentobarbital sodium (50 mg kg^{-1}), a hole 1 mm in diameter, at (Ap = -0.8 mm, L = 1.6 mm, D = 4 mm) was drilled in the skull.

Through this hole, a 25 μL cell suspension ($\sim1\times10^6$ viable cells) was injected into the lateral ventricle slowly over 5 min using a Hamilton syringe, at stereotaxic coordinates (Bai *et al*., 2003).

Spinal cord lesions: All surgical procedures are performed after the animals had been anesthetized by inhaling diethylether. A laminectomy was performed to expose the thoracic spinal cord at levels T_8 through T_9. Iridectomy scissors and a number 11 scalpel blade were used to make an over-hemisection of the spinal cord at the T_8 level, as described previously (Hayashi *et al*., 2009). This lesion was found to have destroyed the bilateral posterior columns and the left lateral and anterior funiculi. After closing the surgical wounds, animal was allowed to recover and were housed. Meinual bladder expression was performed daily until reflex bladder emptying was reestablished. All SCI mice showed monoplegia of the right hindlimbs (Hayashi *et al*., 2009).

Groups

Controls (N = 8): For baseline measurement in this group on the right side an operation was performed which exposed the spinal cord but did not disturb it (Just for induced stress effect of operation).

Spinal cord lesion (N = 8): In this group after operation the right side of spinal cord was crushed.

Spinal cord lesion + cell transplantation (N = 8): In this group after spinal cord lesion cell suspension was injected into the lateral ventricle slowly at stereotaxic coordinates.

Sampling: At the selected post-operative time (4 weeks), rats were anesthetized and intracardially perfused with formaldehyde. Immediately following perfusion a block of the spinal cord segments T_8 to T_9 (approximately 8 mm length) was removed. The spinal blocks were placed in the same fixative for post sampling fixation overnight and then processed and embedded in paraffin. The blocks were sectioned serially at 7 μm. A uniform random sampling scheme was employed so that about 10 sections from each block were sampled. With each section thus selected its immediately preceding neighbor was also collected. Sections were stained with toluidine blue staining method with special buffer of acetic acid, sodium acetate and distilled water (pH = 4.65). After permanent mounting the number of motoneurons in right sides of ventrolateral regions of the spinal cord ventral horns (T_8 to T_9) were determined, using stereological counting technique; physical dissector (Behnam-Rasouli *et al*., 2000).

Statistical analysis: The ratio of numerical density of neurons in samples of spinal cord was as Mean±SE then used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test.

RESULTS

To characterize $BMSC_s$ in the short-term after transplantation, rats were transplanted with $BMSC_s$ immediately after injury and sacrificed at 28 days to examine the time course of cell migration and fate.

The effects of cell transplantation treatment on the numbers of intact neurons in the right ventral horn of spinal cord region at 28 days after spinal cord injury in rats are shown in Fig. 2. The control group revealed healthy neuronal cells amounted by $11000\times10^{-9}\pm400$ Intact neurons (Fig. 2) and spinal cord crush resulted in massive neuronal damage manifested as a significant ($p<0.05$) 50% decrease in the number of normal appearing neurons ($5850\times10^{-9}\pm150$) (Fig. 1, 2).

The result of cell trophy in animal with spinal cord injury show that in this group the number of normal neuron increased in compare with spinal cord injury group ($7800\times10^{-9}\pm256$). Although, with cell trophy the rate of degeneration was decreased but still a significant ($p<0.05$) decrease in the number of intact neurons when compared to the control group has remained.

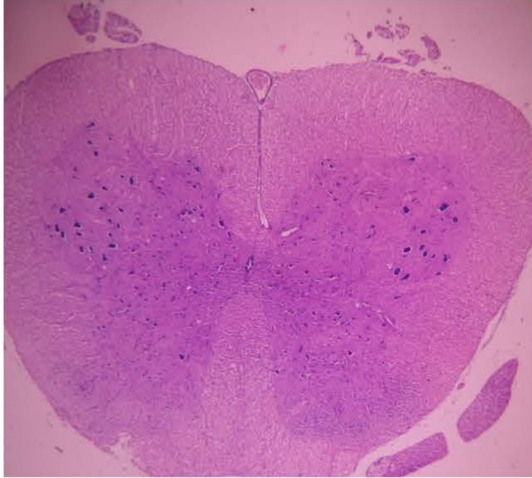

Fig. 1: Photomicrographs illustrate neurons within the anterior horn of spinal cord stained with tuliedin blue and eosin at magnification of (20x) 28 days after spinal cord injury (Right: SCI Left:sham)

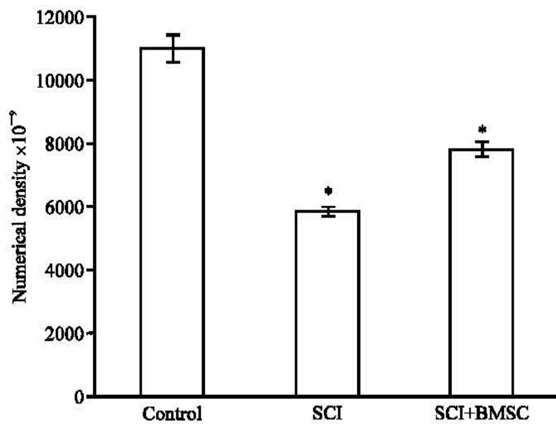

Fig. 2: Effects of Spinal Cord Injury (SCI) and cell administration on the number of intact neurons right ventral horn of spinal cord in rat. Results are expressed as Mean±SD of 8 rats and data were analyzed by one-way ANOVA followed by Tukey-kramer multiple comparisons test. Significantly difference (*p<0.05) exist between control, spinal cord injury (SCI), spinal cord injury (SCI) + cell administration

DISCUSSION

The present study showed that neural stem cells could be wildly disseminated and proliferates in the spinal cord by injection into the lateral ventricle and that when the spinal cord was injured; the attached cells migrated and were integrated into the injured spinal cord tissue. This indicates that injection of neural stem cells through the CSF is a practical method to graft cells into traumatic as well as diseased lesions of the spinal cord. This finding is coordinate with other researcher finding (Bai *et al*., 2003).

The numerical density of SCI group is show a remarkable decreased in density ($p<0.05$). It means that after spinal cord injury some of the neurons were degenerated because of this process the numerical density is decreased in compare with control group.

Result show that cell trophy could repair this process. Numerical density in this group has a significant increase in intact or normal neurons in compare with SCI group ($p<0.05$). As in Fig. 2 is shown numerical density in group (SCI+BMNS) is very near to control group. So at the point of result, all finding indicate that administration of cell in animal has a benefic effect for reducing the rate of degeneration process that occurs after spinal cord injury (Garbossa *et al*., 2006). It is clear that Stem cells, in the inches around the ependymal layer proliferate after neuronal loss. This proliferation is triggered off by the various messenger cascades provoked by the injury (Ao *et al*., 2007). The proliferation however is predominantly astroglial. One of the mechanisms by which steroids and immune modulating drugs aids the functional recovery after spinal cord injury is by suppressing the messenger cascades, thus limiting gliosis. Gliosis in most situations impairs attempts of axons to regrow and reestablish communications (Belegu *et al*., 2007).

Researchers previously demonstrated that implanted DC_s in the injured spinal cord exerted eurotrophic effects, including activation of the endogenous NSPCs and induction of neurogenesis (Blesch *et al*., 2002).

NSPCs constitutively secrete various neurotrophic factors, including BDNF and the implantation of NSPCs has been shown to promote functional recovery in a rat SCI model (Lu *et al*., 2003).

Furthermore, GM-CSF treatment increased the expression of BDNF, an important neurotrophic factor for the treatment of SCI, in injured spinal cord. Recently, GM-CSF has been shown to induce BDNF expression in microglia in a rat SCI model (Bouhy *et al*., 2006). As described above, $NSPC_s$ have the ability to secrete neurotrophins (Lu *et al*., 2003).

In the setting of spinal cord injury therefore, stem cell therapy could potentially be in the following forms:

- Stimulating the endogenous stem cell population to proliferate along neuronal lines. This stimulating cause the cells to migrate from around the control canal along tracts similar to the one provided by the radial glia (Moreno-Manzano *et al*., 2009) in the

cortex. They would serve to replenish the anterior horn cells injured at the level of injury
- Controlling the switches or messengers, for example using sonic hedge hog to push neural stem cells along neuronal lines would augment the process. However, replenishing anterior horn cells at injury levels would only produce a small quantum of clinical benefit

In addition mesenchymal stromal cells have been shown to facilitate axonal regeneration in the rat model. This can occur due to the genesis of oligodendrocyte precursors. It can also explained by the scavenger and immunomodulatory effect of these multipotential cells (Vroemen *et al*., 2003).

The neural stem cells were conveyed in the CSF through the median and lateral apertures into subarachnoid apace of the spinal cord. In the course of flowing down in the CSF, neural stem cells became attached to the pia mater of the spinal cord from the cervical to the sacral, level. The high viability and proliferation of the injected neural stem cells on the spinal cord surface indicate that CSF provides a beneficial environment for cell survival and growth (Parr *et al*., 2008).

The literature over the past few decades clearly documents a growing appreciation of the very complicated pathophysiology of SCI. During this time, the field of stem cell biology has emerged (McDonald *et al*., 2004). This new field has the potential to bring therapies to previously untreatable diseases and injuries (Vaquero and Zurita, 2009).

In total, our finding show that injection stem cell into lateral ventricle in brain after spinal cord injury was reduced the rate of degeneration process and numerical density in this group is very near to control group.

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