Cell Therapy in Spinal Cord Injury: a Mini- Reivew

Soraya Mehrabi [1,2], Sanaz Eftekhari [1,2],bFateme Moradi[1,3], Hamdollah Delaviz [1,4], Bagher Pourheidar [1,5], Monir Azizi [1,3], Adib Zendehdel[1,3], Ali Shahbazi [6], Mohammad Taghi Joghataei*[1]

1. Division of Neuroscience, Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Neuroscience, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Anatomy, School of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran.
5. Department of Anatomy, School of Medicine, Urmieh University of Medical Sciences, Urmieh, Iran.
6. Physiology Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Article info:
Received: 06 August 2012
First Revision: 17 August 2012
Accepted: 12 March 2013

Key Words:
Spinal Cord Injury,
Stem Cell,
Regeneration,
Olfactory Mucosa,
Schwann Cells,
Bone Marrow Stromal Cells,
Stromal Derived Factor.

ABSTRACT

Spinal cord injury (SCI) is a debilitating disease which leads to progressive functional damages. Because of limited axonal regeneration in the central nervous system, there is no or little recovery expected in the patients. Different cellular and molecular approaches were investigated in SCI animal models. Cellular transplantation of stem cells can potentially replace damaged tissue and provide a suitable microenvironment for axons to regenerate. Here, we reviewed the last approaches applied by our colleagues and others in order to improve axonal regeneration following SCI. We used different types of stem cells via different methods. First, fetal olfactory mucosa, schwann, and bone marrow stromal cells were transplanted into the injury sites in SCI models. In later studies, was applied simultaneous transplantation of stem cells with chondroitinase ABC in SCI models with the aid of nanoparticles. Using these approaches, considerable functional recovery was observed. However, considering some challenges in stem cell therapy such as rejection, infection, and development of a new cancer, our more recent strategy was application of cytokines. We observed a significant improvement in motor function of rats when stromal derived factor-1 was used to attract innate stem cells to the injury site. In conclusion, it seems that co-transplantation of different cells accompanies with other factors like enzymes and growth factors via new delivery systems may yield better results in SCI.

1. Introduction

The trend in the major causes of death has changed over the past years from infectious diseases to cardiovascular diseases, cancers and road traffic accidents. Road traffic accident is one of the main causes of Spinal Cord Injury (SCI), a debilitating disease, which leads to progressive functional damages. It is estimated that the annual incidence of spinal cord injury is approximately 50 per 1 million people (Ackery, Tator, & Krassioukov, 2004). Prevalence of traumatic SCI in Tehran ranged from 1.2 to 11.4 per 10,000 people (Rahimi-Movaghar et al., 2009).There are about 89000 patients suffering from this problem in Iran, 66% of them caused by road traffic accidents (Joghataei, 2009). SCI represents an injury with catastrophic outcomes, both for the individual on a personal level, and for society with respect to the extent of burden on the health care and living expenses (Kwon, Sekhon, & Fehlings, 2010). Although, considerable research efforts were undertaken, only limited rehabilitating therapies are available in human patients. Unlike the peripheral nervous system (PNS), the adult mammalian the CNS have limited capacity to spontaneously regeneration following an injury. The Edwin Smith surgical papyrus, which dates back to 1550 BC, states that "If you examine a man with a neck injury … and find he is without sensation in

**** Corresponding Author:***
Mohammad Taghi Joghataei, PhD
Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran.
Tel/Fax:021- 88622578
E-mail: joghataei@tums.ac.ir

both arms and both legs, and unable to move them, and he is incontinent of urine …it is due to the breaking of the spinal cord caused by dislocation of a cervical vertebra. This is a condition which cannot be treated (Filbin, 2003). Many approaches were used by investigators in order to facilitate axonal regeneration and consequent functional recovery following SCI. Here, we review briefly different strategies applied by researchers and our colleagues in animal models of SCI.

2. Inhibitors of Axonal Regeneration in the Adult CNS

Injury to the CNS induces tissue damage, which creates barriers to regeneration. One of the main barriers is the glial scar, in which astrocytes and some other cells establish a dense cellular response surrounding the lesion site (Dawson, Levine, & Reynolds, 2000; P. Lu, Jones, & Tuszynski, 2007; Silver & Miller, 2004). These cells express several inhibitory molecules including chondroitin sulfate proteoglycans (CSPGs) and keratan sulfate proteoglycans (KSPGs) which fill the extracellular matrix (ECM) surrounding the lesion site (Jones & Tuszynski, 2002; Tang, Davies, & Davies, 2003). The astrocyte response to injury is referred to as reactive gliosis (Silver & Miller, 2004). As axons cannot regenerate beyond the glial scar, this extracellular glial scar is thought to be a major limiting factor following CNS injury (Grimpe & Silver, 2002; Silver & Miller, 2004); However, increasing evidences suggests a beneficial role of this scar tissue in reestablishing the physical and chemical integrity of the CNS. Another event which makes more complex the regeneration process is progressive cavitation in which, after days to weeks, a CNS injury can expand in size; leading to a scar-encapsulated cavity more expanded the size of initial lesion (Balentine, 1978; Fitch, Doller, Combs, Landreth, & Silver, 1999).

3. New Approaches to Spinal Cord Injury Treatment

Recently, basic scientists have being applied several strategies aimed to provide new treatments (Barnett & Riddell, 2004). These include (a) promoting the survival and growth of damaged axons using different neurotrophins; (b) neutralizing inhibitory molecules associated with the failure of axonal regeneration, (c) providing a permissive growth environment by transplanting neural cells, and (d) gene therapy (Bamber et al., 2001; Bradbury et al., 2002; Qiu et al., 2002); (Harrop, Poulsen, Xiao, Freese, & During, 2004; Poulsen, Harrop, & During, 2002) .

4. Challenges of Cell Therapy for Spinal Cord Injury

Stem cells can be classified into two major categories, (i) Embryonic stem cells (ES) are pluripotent stem cells capable of differentiate to most tissues of the organism. Human ESCs are derived from discarded, non-transferred human embryos, from the inner

cell mass of a blastocyst using an immunosurgical technique. (ii) Adult stem cells are undifferentiated cells found throughout the body that divide to replenish

dying cells and regenerate damaged tissues. Adult stem cells can be isolated from a tissue sample obtained from an adult organ. Compared to adult stem cells ES cells are clinically more effective for neurological disorders. (Paspala et al 2009)

4.1. Olfactory Ensheathing Cell Transplantation

A range of cells have been investigated for use in transplantation; These include neural stem cells and glial cells such as olfactory ensheathing cells, Schwann cells and oligodendrocyte precursor cells (Barnett & Riddell, 2004). One of the most favorable candidates for cellular transplant-mediated repair of CNS lesions is the olfactory ensheathing cells (OEC). OECs reside in the olfactory system, which supports neurogenesis throughout the life (Farbman, 1990).The olfactory system originates from the olfactory placode and is made up of olfactory epithelium (PNS tissue) and olfactory bulb (CNS tissue) (Barnett & Riddell, 2004). After injury or during normal cell turnover, new olfactory receptor neurons (ORNs) are generated from the basal stem cells in the olfactory epithelium, which extend axons through the cribriform plate and re-enter the olfactory bulb, re-synapsing with second-order neurons in the glomerular layer (Schwob, 2002). This is one of the rare situations in which peripheral axons are able to enter the adult CNS environment and form synapses, and it is thought that this unusual ability may be due in part to unique specialized properties of OECs (Farbman, 1990). In a study by Raisman et al, the upper cervical corticospinal tract was transected on one side in adult rats; then a suspension of ensheathing cells cultured from adult rat olfactory bulb was injected into the lesion site; this induced unbranched, elongative growth of the transected corticospinal axons. The axons grew through the transplant and continued to regenerate into the denervated caudal host tract. Rats with complete transections and no transplanted cells did not use the forepaw on the lesioned side for directed reaching; while, Rats in which

the transplanted cells had formed a continuous bridge across the lesion exhibited directed forepaw reaching on the lesioned side (Li, Field, & Raisman, 1997).

In most previous studies, transplantation was carried out at the same time the lesions were made. The potential benefit of such an approach to human spinal cord injuries in clinical situation should take in account the unavoidable time delay between the time of injury and the time of transplantation. This issue leaded the researchers to work on delayed transplantation. For example, Lu et al, and Plant et al, reported an improvement of locomotor function following delayed transplantation of OECs into complete or incomplete thoracic spinal lesions (J. Lu, Feron, Mackay-Sim, & Waite, 2002; Plant, Christensen, Oudega, & Bunge, 2003). In a study by Naghmeh Keyvan-Fouladi et al, destruction of the dorsal corticospinal tract on one side at the level of the first cervical segment abolished the use of the ipsilateral forepaw for retrieval for at least 6 months after operation; in lesioned rats that had shown no retrieval for 8 weeks after operation, a suspension of olfactory ensheathing cells was injected into the lesion site; starting between 1 and 3 weeks after transplantation, all rats with transplants bridging the lesion site resumed retrieval by the ipsilateral forepaw; furthermore, by biotin dextran anterograde tracing they also showed regenerating corticospinal axons crossing the bridge, traveling caudally for about 10mm in the distal part of the corticospinal tract and forming terminal arborizations in the spinal gray matter. They provide an assay for determining the effectiveness of different methods of cell preparation or different cell types (Keyvan-Fouladi, Raisman, & Li, 2003). In another study by same group, it was reported that transplantation of cultured adult peripheral nerve schwann cells also restores function, but the effect is delayed until around 30 days after transplantation and reaches only around 5-10% of normal (Keyvan-Fouladi, Raisman, & Li, 2005)

4.2. Fetal Olfactory Mucosa Transplantation

Unlike rats, the olfactory bulb in humans is relatively small and inaccessible (Franklin, 2002). Since transplantation of fetal olfactory mucosa (FOM) is more safe and feasible, in one study we investigated the possible beneficial effects of FOM transplantation on the recovery of locomotor function and also spinal tissue sparing following spinal cord hemisection (Delaviz et al., 2008). Adult female rats were spinally hemisected at the L1 level and were randomized into the three groups. The first group was immunosuppressed injured animals received Cyclosporine A (CsA) and FOM graft. The second group received CsA and fetal respiratory mucosa (FRM) graft, and the control group, non-immunosuppressed rats, received saline and gel foam. From weeks 6-8, the functional recovery of the FOM rats significantly increased in comparison to the FRM, although a significant difference in tissue sparing was not apparent. From weeks 2-8, the functional recovery of the FOM and FRM groups as well as tissue sparing of the FOM group increased significantly compared to the control group .This study showed that transplantation of fetal olfactory mucosa with its lamina prafopria and olfactory neuroepithelium results in promotion of tissue sparing and functional recovery in mammals with partial spinal cord injury.

4.3. Bone Marrow Stromal Cells and Schwann Cell Transplantation

Different studies indicate that bone marrow stromal cells (BMSCs), can differentiate into adipocytes; chondrocytes; and osteocytes after transplantation in mice and rats (Pittenger et al., 1999). Oligodendrocytes, are less in number compare to neurons, and are able to express the markers of these cells (Steidl et al., 2002). They can promote injured tissue repair by reducing cavity formations caused by tissue necrosis in the site of lesions (Wu et al., 2003). BMSCs create a suitable axonal growth environment through the expression of growth factors such as neurotrophins (Chen et al., 2002; Mahmood, Lu, Wang, & Chopp, 2002). They can also improve vascularization leading to repair of damaged tissue (Mahmood et al., 2002).

Another cell type that has been used for repair of 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 the area of spinal cord lesion (Bunge, 2006; Pearse & Barakat, 2006) . SCs produce neurotrophic factors and cell adhesion molecules leading to promotion of axonal growth (Oudega & Xu, 2006). They also suppress the cavity formation when transplanted into the injured spinal cords (Pearse et al., 2007; Takami et al., 2002). Studies have shown that SC transplantation significantly improves tissue sparing and results in an increase in the number of myelinated axons in implantation areas (Takami et al., 2002). It has been reported that SC injection into the contused spinal cord promotes myelination and regeneration of supraspinal axons and therefore improves locomotor recovery (Takami et al., 2002). The advantage of the SC compared to other commonly used cells in the cell therapy is the superior ability of this cell to myelinate the demyelinated axon (Oudega, Moon, & de Almeida Leme, 2005).

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 (Takami et al., 2002) .Since a combination-therapy strategy seems more promising, we investigated whether co-transplantation of these cells can improve treatment outcome (Joghataei Mohammad Taghi., 2010) . In our study, contusion model of SCI was created at the T8-9 level in adult male rats. BMSCs and SCs were cultured and prelabeled with BrdU and 1,1' dioctadecyl 3,3,3',3' tetramethylindocarbocyanin perchlorate respectively. The rats were divided into seven groups. These groups included: a control group, three experimental groups and three sham groups. In the control group only 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, or combination of BMSCs and SCs, respectively. The sham groups received serum in the same manner. 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. It was showed that BrdU-positive BMSCs, transplanted in the site of injury, survived and reorganized around the cavity center. Fluorescence microscopy also shows the presence of S-100-positive cells at the site of injury, confirmed that intra-spinaly transplanted SCs survived for a long time. 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. In functional assessment, 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; in contrast, the statistical difference between SC and BMSC groups was not significant.

5. Intra-Thecal Delivery of Chemokines

Considering potential complications of cell transplantation such as rejection; infection; and development of new cancer our recent approach was using chemokines like stromal-derived factor-1 (SDF-1) , cytokines like granulocyte colony-stimulating factor (G-CSF) and other factors for attracting and mobilizing innate stem cells toward the injury site. In a study by Kawada et al, it has been shown that G-CSF mobilizes bone marrow-derived stem cells to repair the ischemic myocardium (Kawada et al., 2004). In an experiment by Koda et al, they showed that G-CSF mobilizes bone marrow-derived cells into injured spinal cord and promotes functional recovery after compression-induced spinal cord injury in mice (Koda et al., 2007); and recently we tested the effect of SDF releasing pump on functional recovery in experimental model of spinal cord injury in rats. In this study, Brdu immunohistochemistry, and BBB tests showed a significant improvement in treated rats (Zendedel et al., 2012)

6. Self-Assembling Peptides

One of the effective strategies for spinal cord regeneration is the transplantation of scaffold contained stem cells to the lesion site; because implanted cells accompanied by scaffold may act as a bridge and also as secretors of pre-regenerative molecules such as growth factors. Different members of a self-assembling peptide hydrogel family have been evaluated as three-dimensional (3D) culture systems for carrying different cells. BD PuraMatrix peptide hydrogel, a three-dimensional cell culture model of nanofiber scaffold derived from the self-assembling peptide RADA16, can be applied to regenerative tissue repair in order to develop novel nanomedicine systems. In another study by our group, self-assembling nano-fiber scaffold (SAPNS) and schwann cells isolated from human fetal sciatic nerves transplanted into the spinal cord after injury in rats. Immunohistochemical analysis of grafted lumber segments at 8 weeks after grafting revealed reduced asterogliosis and considerably increased infiltration of endogenous S-100 positive cells into the injury site; suggesting that PuraMatrix plays an important role in the repair observed following transplantation of SAPNS with human fetal SC (Moradi et al, 2012)

7. Conclusion

In conclusion, cell therapy is a promising strategy in the field of SCI treatment. However, the approaches of cell therapy are an ever changing field. It seems that co-transplantation of different cells accompanies with other factors like enzymes and growth factors via new delivery systems may yield better results in SCI.

References

Ackery, A., Tator, C., & Krassioukov, A. (2004). A global perspective on spinal cord injury epidemiology. J Neurotrauma, 21(10), 1355-1370.

Balentine, J. D. (1978). Pathology of experimental spinal cord trauma. I. The necrotic lesion as a function of vascular injury. Lab Invest, 39(3), 236-253.

Bamber, N. I., Li, H., Lu, X., Oudega, M., Aebischer, P., & Xu, X. M. (2001). Neurotrophins BDNF and NT-3 promote axonal re-entry into the distal host spinal cord through Schwann cell-seeded mini-channels. Eur J Neurosci, 13(2), 257-268.

Barnett, S. C., & Riddell, J. S. (2004). Olfactory ensheathing cells (OECs) and the treatment of CNS injury: advantages and possible caveats. J Anat, 204(1), 57-67, 10.1111/j.1469-7580.2004.00257.x.

Bradbury, E. J., Moon, L. D., Popat, R. J., King, V. R., Bennett, G. S., Patel, P. N., et al. (2002). Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature, 416(6881), 636-640, 10.1038/416636a.

Bunge, P. W. (2006). Textbook of Neural Repair and Rehabilitation: Cambridge.

Chen, X., Katakowski, M., Li, Y., Lu, D., Wang, L., Zhang, L., et al. (2002). Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. J Neurosci Res, 69(5), 687-691, 10.1002/jnr.10334.

Dawson, M. R., Levine, J. M., & Reynolds, R. (2000). NG2-expressing cells in the central nervous system: are they oligodendroglial progenitors? J Neurosci Res, 61(5), 471-479.

Delaviz, H., Joghataie, M. T., Mehdizadeh, M., Bakhtiyari, M., Nobakht, M., & Khoei, S. (2008). Transplantation of olfactory mucosa improve functional recovery and axonal regeneration following sciatic nerve repair in rats. Iran Biomed J, 12(4), 197-202.

Farbman, A. I. (1990). Olfactory neurogenesis: genetic or environmental controls? Trends Neurosci, 13(9), 362-365, 10.1016/0166-2236(90)90017-5.

Filbin, M. T. (2003). Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. Nat Rev Neurosci, 4(9), 703-713, 10.1038/nrn1195.

Fitch, M. T., Doller, C., Combs, C. K., Landreth, G. E., & Silver, J. (1999). Cellular and molecular mechanisms of glial scarring and progressive cavitation: in vivo and in vitro analysis of inflammation-induced secondary injury after CNS trauma. J Neurosci, 19(19), 8182-8198.

Franklin, R. J. (2002). Obtaining olfactory ensheathing cells from extra-cranial sources a step closer to clinical transplant-mediated repair of the CNS? Brain, 125(Pt 1), 2-3.

Grimpe, B., & Silver, J. (2002). The extracellular matrix in axon regeneration. Prog Brain Res, 137, 333-349.

Harrop, J. S., Poulsen, D. J., Xiao, W., Freese, A., & During, M. J. (2004). Effect of altering titer, serotype, and promoter in recombinant adenoassociate virus gene therapy expression of spinal cord neurons and astrocytes. Spine (Phila Pa 1976), 29(24), 2787-2792.

Joghataei Mohammad Taghi., B. M., Pourheydar Bagher.,Mehdizadeh Mehdi.,Faghihi Abolfazl.,Mehraein Fereshteh., Behnam Babak., Pirhajati Vahid.,. (2010). Co-transplantation of Schwann and Bone Marrow Stromal Cells Promotes Locomotor Recovery in the Rat Contusion Model of Spinal Cord Injury. Yakhteh Medical Journal 12(1), 7-16.

Joghataei, M. T. (2009). Disability Prevalence in Iran o. Document Number)

Jones, L. L., & Tuszynski, M. H. (2002). Spinal cord injury elicits expression of keratan sulfate proteoglycans by macrophages, reactive microglia, and oligodendrocyte progenitors. J Neurosci, 22(11), 4611-4624, 20026464.

Kawada, H., Fujita, J., Kinjo, K., Matsuzaki, Y., Tsuma, M., Miyatake, H., et al. (2004). Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood, 104(12), 3581-3587, 10.1182/blood-2004-04-1488.

Keyvan-Fouladi, N., Raisman, G., & Li, Y. (2003). Functional repair of the corticospinal tract by delayed transplantation of olfactory ensheathing cells in adult rats. J Neurosci, 23(28), 9428-9434.

Keyvan-Fouladi, N., Raisman, G., & Li, Y. (2005). Delayed repair of corticospinal tract lesions as an assay for the effectiveness of transplantation of Schwann cells. Glia, 51(4), 306-311, 10.1002/glia.20211.

Koda, M., Nishio, Y., Kamada, T., Someya, Y., Okawa, A., Mori, C., et al. (2007). Granulocyte colony-stimulating factor (G-CSF) mobilizes bone marrow-derived cells into injured spinal cord and promotes functional recovery after compression-induced spinal cord injury in mice. Brain Res, 1149, 223-231, 10.1016/j.brainres.2007.02.058.

Kwon, B. K., Sekhon, L. H., & Fehlings, M. G. (2010). Emerging repair, regeneration, and translational research advances for spinal cord injury. Spine (Phila Pa 1976), 35(21 Suppl), S263-270, 10.1097/BRS.0b013e3181f3286d.

Li, Y., Field, P. M., & Raisman, G. (1997). Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science, 277(5334), 2000-2002.

Lu, J., Feron, F., Mackay-Sim, A., & Waite, P. M. (2002). Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. Brain, 125(Pt 1), 14-21.

Lu, P., Jones, L. L., & Tuszynski, M. H. (2007). Axon regeneration through scars and into sites of chronic spinal cord injury. Exp Neurol, 203(1), 8-21, 10.1016/j.expneurol.2006.07.030.

Mahmood, A., Lu, D., Wang, L., & Chopp, M. (2002). Intracerebral transplantation of marrow stromal cells cultured with neurotrophic factors promotes functional recovery in adult rats subjected to traumatic brain injury. J Neurotrauma, 19(12), 1609-1617, 10.1089/089771502762300265.

Oudega, M., Moon, L. D., & de Almeida Leme, R. J. (2005). Schwann cells for spinal cord repair. Braz J Med Biol Res, 38(6), 825-835, /S0100-879x2005000600003.

Oudega, M., & Xu, X. M. (2006). Schwann cell transplantation for repair of the adult spinal cord. J Neurotrauma, 23(3-4), 453-467, 10.1089/neu.2006.23.453.