Balneo Research Journal DOI: http://dx.doi.org/10.12680/balneo.2016.114 Vol.7, No.1, February 2016

NEURORESTORATION: FUTURE PERSPECTIVE IN REHABILITATION OF CHRONIC SPINAL CORD INJURIES

Ioana Stanescu[1´3], Gabriela B. Dogaru[2´3]

1. University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj – Neuroscience Department
2. University of Medicine and Pharmacy "Iuliu Hatieganu" – Medical Rehabilitation and Physical Medicine Department
3. Rehabilitation Hospital Cluj

ABSTRACT

Traumatic spinal cord injury leads to severe and mostly irreversible neurological deficits that alter physical status and quality of life of affected patients. The goals of classical therapeutical approaches in SCI are to improve functional level, decrease secondary morbidity and enhance health-related quality of life. Neurorestoratology is a new neuroscience discipline that studies replacement of impaired nervous components through neural regeneration, neuroplasticity, neuroprotection and neuromodulation. Over the past years, various neurochemical and cellular repair strategies have been evaluated in experimental models of SCI for their efficacy in promoting neuroplasticity, axon regeneration, remyelination, and re-establishment of spinal circuitry to improve motor recovery following injury. These neurorestorative strategies are safe and feasibl, have good preclinical results, being under way to be translated into clinical studies, enabling patients with chronic SCI to get benefits in the future.

Key words: spinal cord injury, neurorestoration, cell therapy, functional outcome

INTRODUCTION

Traumatic spinal cord injury (SCI) leads to severe and mostly irreversible neurological deficits and motor and sensory dysfunctions that alter the physical, psychical and social status of affected patients. According to the National Spinal Cord Injury Statistical Center (NSCISC) 2015, the incidence of SCI is estimated at 40 cases per million populations in the United States. The average age at injury in now 42 years, 80% are males, and vehicle crashes are the leading cause of injury (1).

The most frequent neurologic syndrome after spinal cord injuries is incomplete tetraplegia, followed by incomplete paraplegia. According to the NSCISC less than 1% of patients suffering a SCI recover completely after the traumatism (1).

Neurorestoratology will became of the most important disciplines in neuroscience; the goal of neurorestoration is to prevent or limit neuronal damage, to promote and maintain the integrity of damaged neural functions, to increase neuronal survival in an unfavorable microenvironment and to minimise glial scar formation. This new discipline studies replacement of impaired nervous components, neural regeneration, neuroplasticity, neuroprotection and neuromodulation, and uses mainly novel cell-based strategies to restore the lost functions (4).

Spinal cord injuries are classified according to the American Spinal Injury Association (ASIA) by considering the motor and sensory functions. The last revision of the ASIA Disorder Scale was made in 2011 (3).

ASIA-A: Complete. There is no sensory or motor function preserved in the sacral segments of S4-S5

ASIA-B: Sensory incomplete. Motor deficit without sensory loss below the neurological level, including the sacral segments of S4-S5 (light touch, pin sensation or deep anal pressure at S4-S5), and there is no protected motor function from three levels below the motor level at each half of the body.

ASIA-C: Motor incomplete. Motor function is preserved below the neurological level and more than half of the muscles below this level have strength lower than 3/5 (0, 1 or 2).
ASIA-D: Motor incomplete. Motor function is preserved below the neurological level and at least half of the muscles (half or more) below this level have strength higher than 3/5.
ASIA-E: Normal. Sensory and motor function as assessed by ISNCSC in all segments are normal and in patients with pre-existing deficits there is "E" degree of ASIA. Initially one without a spinal cord injury does not have an ASIA degree.
The life expectancy for persons with SCI have not improved since the 1980s and remain significantly below that of persons without SCI. Mortality rates are significantly higher during the first year after injury than during subsequent years, particularly for persons with the most severe neurological impairments (NSCISC, 2015) (1).

The goals of rehabilitation and other treatment approaches in SCI are to improve functional level, decrease secondary morbidity and enhance health-related quality of life (2). Treatment of patients with spinal cord injury treatment is an ongoing process for many years and starts shortly after the injury with acute care and early surgical interventions; thereafter, sensory, motor and autonomic dysfunction treatment in the chronic phase and finally, life long treatment in the home environment.

Once the spinal cord lesion occurs, tissue and neurobiological damages evolve over time, causing so called “secondary cord lesion” (4). Understanding the mechanisms of secondary injury after the acute phase of spinal cord injury should provide novel neuroprotective strategies. Recently, a number of studies have shown promising results on neuroprotection and recovery of function in rodent models of spinal cord injury using treatments that target secondary injury processes like inflammation, phospholipase A_2 activation, and manipulation of the PTEN-Akt/mTOR signaling pathway (5).

Mechanical injuries cause necrosis of those neurons directly affected by the force of impact (primary injury phase). Acute spinal cord injury initiates a complex cascade of molecular events: ‘secondary injury’, which leads to progressive degeneration ranging from early neuronal apoptosis at the lesion site to delayed degeneration of intact white matter tracts, and, ultimately, expansion of the initial injury.

A secondary injury phase is characterised by a protracted neuronal loss driven by changes in oxygen, glucose, neuroactive lipids and eicosanoids homeostasis, by the release of free radicals and biogenic amines, endogenous opioids and excitatory amino acids. These secondary injury processes include inflammation, free radical-induced cell death, glutamate excitotoxicity, phospholipase A2 activation, and induction of extrinsic and intrinsic apoptotic pathways.

The secondary injury process can be divided temporally into multiple contiguous phases: the immediate, acute, intermediate, and chronic stages of SCI (6). The chronic phase begins ~ 6 months following injury and continues throughout the lifetime of the patient with SCI.

The chronic phase is characterized by continued scar formation, Wallerian degeneration of injured axons and the development of cysts and/or syrinxes (6). It can be considered that at ~ 1-2 years postinjury, the neurological deficits have stabilized and the lesion has fully matured. The lesion itself is characterized by cystic cavitation and myelomalacia, representing the final stage of necrotic death after SCI. The clinical status could remain stable or aggravations could appear caused by syrinx formation (in 30% of patients), manifested by increasing motor deficit, brainstem symptoms or neuropathic pain.

At this chronic stage, therapeutic strategies are aimed to encourage regeneration/sprouting of disrupted axons, promote plasticity with rehabilitation strategies, and improve the function of demyelinated axons with

pharmacological measures or cellular transplantation substrates that may potentially remyelinate. (6). Cellular and pharmacological approaches have been developed following spinal cord injury in animal models. Over the past years, various neurochemical and cellular repair strategies have been evaluated in experimental models of SCI for their efficacy in promoting neuroplasticity, axon regeneration, remyelination, and re-establishment of spinal circuitry to improve motor recovery following injury.

Unfortunately, the restoring capacity of the damaged spinal cord is very limited because of reduced intrinsic growth capacity and non-permissive environment for axonal elongation. There are 2 distinct aspects to regenerative failure after CNS injury—the limited intrinsic regenerative potential and the inhibitory extrinsic environment of the injured CNS. (6). The regenerative processes are blocked by diverse intrinsic factors such as growth inhibitory proteins and the glial scar formed in the site of lesion. Neurons do regenerate if a permissive environment is provided. Neurotrophic support is also necessary to achieve spinal cord's restorative ability.

Neurorestorative strategies involve pharmacologic techniques, cell therapies, gene therapies and tissue engineering.

I. Pharmacological / molecular regeneration and repair strategies.

For these therapeutic approaches researchers need to identify molecular signals required to stimulate axonal growth following injury. These molecules are divided into inhibitors associated with CNS myelin and inhibitors associated with the astrocytic glial scar. Several regeneration-associated genes have been identified: *L1*, *c-fos*, and *c-jun*, and the 43 kD growth-associated protein (7), (8). The elevation of intracellular cAMP levels using cAMP analogs or the phosphodiesterase inhibitor rolipram has also been shown to increase axonal sprouting and reduce the effects of myelin-associated inhibitors (9). Rho/ROCK kinase system is specifically activated by the components of damaged spinal cord tissue, including oligodendrocytes and myelin, as well as extracellular matrix.

Also, identification of inhibitory molecules that make the injured CNS an inhibitory environment for axonal growth is of crucial importance in developing restorative pharmacological strategies. Two types of inhibitory molecules have been identified: myelin-associated inhibitors and inhibitors associated with the astrocytic glial scar.

I.1. Myelin-associated inhibitors: multiple components of central nervous system myelin inhibits axonal regrowth (Nogo, MAG, OMgp, semaphorin 4D, ephrin B3, repulsive guidance molecule, Netrin-1), by inducing collapse of growth cones on regenerating axons (6). All known myelin inhibitors appear to activate the guanosine triphosphatase Rho, which leads to growth cone collapse.

The use of anti-Nogo antibodies in a rat model of partial SCI was found to promote axonal regeneration and functional recovery (10), (11).

Rho-ROCK inhibition promoted axonal sprouting and improved locomotor function in an incomplete thoracic transection model of SCI in the mouse (12). The administration of C3 enzyme from *Clostridium botulinum* (which selectively inactives Rho) inhibits the apoptotic cell death (13). A C3-like enzyme was used in human studies under the commercial name of Cethrin, and and has shown encouraging results (14).

I.2. Glial scar-associated inhibitors: The glial scar is the result of reactive astrocytosis and is a constant response to all CNS injuries. The astrocytes that form the scar secrete a number of growth inhibitory extracellular matrix components known as CSPGs (15). The main strategy for overcoming this glial barrier is by direct degradation of the CSPGs with a bacterial enzyme known as chondroitinase ABC

(ChABC) (16), (17). Numerous investigators are using ChABC in combination with cell transplantation therapies, in order to permeabilise the glial scar.

II. **Cell transplantation therapies** have become a major component of experimental and clinical research as a promising strategy for the treatment of chronic spinal cord injury. The transplanted cells are supposed to replace lost neurons or olygodendrocytes, to remyelinate danaged axons or to promote a permissive microenvironment for neuroregeneration. Various cell types have different functions in injured-cord environment: some cells have potential to form myelin, others promote and guide axonal growth, bridging the site of injury, and others secrete neurotrophic factors. They have been many pre-clinical studies of cell transplantations over the past decades. The most realistic goal of these therapies remain remyelination of injured demyelinated axons.

Routes of cell transplantation used in human studies are (4): intraspinal cord transplantation, cerebellomedullary cistern transplantation, cervical, thoracic or lumbar subarachnoid space transplantation and intravenous transplantation.

The most common cell types which were used in experimental studies include Schwann cells, olfactory ensheathing glial cells, embryonic and adult neural stem/progenitor cells, fate-restricted neural/glial precursor cells, and bone-marrow stromal cells (18).

II.1. Schwann cells: Implantation of Schwann cells through a peripheral nerve tissue graft could theoretically support the axonal growth. On experimental contusive SCI model in the rat, Xu at al (19) have shown that Schwann cell transplantation can result in enhanced axonal regeneration and remyelination, which was associated with a very small, statistically significant, improvement in hindlimb function. Schwann cells have a proven ability to integrate into the injured cord parenchyma, and could also be used as a delivery mechanism for other therapeutic molecules such as growth factors.

II.2. Olfactory unsheathing cells (OEC) are specialized glial cells, that *in vivo* have the ability to facilitate the passage of axons from peripheral nervous system (olfactory receptor neurons) into the hostile environment of the central nervous system (mitral neurons from the olfactory bulb). But the ability of the OEC to myelinate in vivo after transplantation into the injured spinal cord remains a subject of controversy, because evidence indicate that the OECs, in fact, do not form myelin (20), but are able to produce an environment permissive for axonal growth by secretion of neuroprotective and angiogenetic factors, and by modifying the glial scar (21).

II.3. Embrionic brain stem cells (ESCs) are pluripotent cells, derived initially from murine embrios. These cells require in vitro differentiation towards a neural or glial cell line prior to transplantation (4), (6). Experimental studies have proved that production of oligodendroglial lineage cells from ESCs is followed by myelination. The production of a high-purity population of oligodendroglial progenitor cells (OPC) from human ESCs was first achieved by Hans Keirstead and coworkers; they have obtained remyelination and significantly improved locomotor function when transplanted 1 week after SCI in the rat (22), (23).

II.4. Neural stem cells / neural progenitor cells are multipotent cells capable of producing all 3 neural lineages (neurons, astrocytes, and oligodendrocytes). The direction of differentiation and maturity can be influenced by a variety of environmental factors. The transplanted cells have been shown to survive for at least 5 weeks if inserted by lumbar puncture in the syrinx cavity (24). In experimental studies cells migrate up to 15 mm within the spinal cord, and can favor remyelination and increase in motor function (25). Neural stem cells can survive more if transplanted in combination with growth factors

and anti-inflammatory drugs. If transplanted with Schwann cels into the injured spinal cord can promote functional recovery (25), (26).

Adult-derived neural stem cells are self-renewing stem cells that can be isolated in adult humans from the subventricular zone by endoscopic neurosurgical procedures (27).

II.5. Bone marrow stromal cells and hematopoietic stem cells have been reported to have the ability to differentiate into neural lineage cells in appropriate in vitro and in vivo conditions (28). Their advantages are easy access, easy in vitro amplification and possibility to be grafted intramedullary or by intravenous injection, offering the potential for autologous transplantation. The transplantation of marrow stromal cells has been shown to induce remyelination in the demyelinated spinal cord (29).

II.6. Umbilical cord blood cells can differentiate into neural cells undes appropriate induction conditions, having the same therapeutical potential in SCI as the bone marrow stromal cells (30).

Other important neurorestorative techniques that are under development are gene therapies and tissue engineering, and these approaches will be the subject of a following article.

In summary, the pessimistic view that spinal cord injuries lead to irreversible disabilities is slowly disappearing in the light of novel cell transplantation, gene therapies and bioengineering techniques. Cell transplantation therapy is safe and feasible and can can improve partially functional recovery and quality of life in patients with chronic SCI. These neurorestorative strategies have good preclinical results which are under way to be translated into clinical studies, enabling patients with chronic SCI to get benefits in the future.

BIBLIOGRAPHY

1. National Spinal Cord Injury Statistical Center, Facts and Figures at a Glance. Birmingham, AL: University of Alabama at Birmingham, 2015.
2. Nebahat Sezer, Selami Akkuş, Fatma Gülçin Uğurlu. Chronic complications of spinal cord injury. World J Orthop. 2015 Jan 18; 6
3. Kirshblum SC, Burns SP, Biering-Sorensen F, Donovan W, Graves DE, Jha A, Johansen M, Jones L, Krassioukov A, Mulcahey MJ, et al. International standards for neurological classification of spinal cord injury (revised 2011) J Spinal Cord Med. 2011;34:535–546.
4. M'rabet Abdelfatteh, Bouabdallah Mohamed, Ben Rhouma Moncef, Daghfous Mohamed Samir, Mestiri Mondher, Lin Chen, Hongyun Huang, Wagih El Masri, Haitao Xi, Andrey Bryukhovetskiy, Elena Chernykh, Vyacheslav Stupak, Gakhongir Muradov, Mikhail Siziko, Ekaterina Shevela, Olga Leplina, Marina Tikhonova, Alexandr Kulagin, Alexandr Ostanin. Spinal cord injury, In: Neurorestoratology, Volume 2, Editors: H Huang, G Raisman, PR Sanberg et al. 2015, Nova Science Publishers
5. Nai-Kui Liu, Xiao-Ming Xu. Neuroprotection and its molecular mechanism following spinal cord injury. Neural Regen Res. 2012 Sep 15; 7(26): 2051–2062.
6. James W. Rowland, Gregory W.J. Hawryluk, Brian Kwon, Michael G. Fehlings. Current Status of Acute Spinal Cord Injury Pathophysiology and Emerging Therapies: Promise on the Horizon. Neurosurg Focus. 2008;25(5):E2
7. Jenkins R, Tetzlaff W, Hunt SP: Differential expression of immediate early genes in rubrospinal neurons following axotomy in rat. Eur J Neurosci 5:203-209, 1993
8. Chaisuksunt V, Zhang Y, Anderson PN, et al: Axonal regeneration from CNS neurons in the cerebellum and brainstem of adult rats: correlation with the patterns of expression and distribution of messenger RNAs for L1, CHL1, c-jun and growthassociated protein-43. Neuroscience 100:87-108, 2000
9. Hannila SS, Filbin MT: The role of cyclic AMP signaling in promoting axonal regeneration after spinal cord injury. Exp Neurol 209:321-332, 2008
10. Schnell L, Schwab ME: Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. Nature 343:269-272, 1990
11. Bregman BS, Kunkel-Bagden E, Schnell L, et al: Recovery from spinal cord injury mediated by

antibodies to neurite growth inhibitors. Nature 378:498-501, 1995
12. Dergham P, Ellezam B, Essagian C, Avedissian H, Lubell WD, McKerracher L. Rho signaling pathway targeted to promote spinal cord repair. J Nerurosci 2002;22(15):6570-6577
13. Dubreuil CI, Winton MJ, McKerracher L: Rho activation patterns after spinal cord injury and the role of activated Rho in apoptosis in the central nervous system. J Cell Biol 162:233-243, 2003
14. Hawryluk GW, Rowland J, Kwon BK, Fehlings MG. Protection and repair of the injured spinal cord: a review of completed, ongoing, and planned clinical trials for acute spinal cord injury. Neurosurg Focus. 2008;25(5):E14.
15. McKeon RJ, Schreiber RC, Rudge JS, et al: Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. J Neurosci 11:3398-3411, 1991
16. Barritt AW, Davies M, Marchand F, et al: Chondroitinase ABC promotes sprouting of intact and injured spinal systems after spinal cord injury. J Neurosci 26:10856-10867, 2006
17. Garcia-Alias G, Lin R, Akrimi SF, et al: Therapeutic time window for the application of chondroitinase ABC after spinal cord injury. Exp Neurol 210:331-338, 2007
18. Wolfram Tetzlaff, Elene Okon, Soheila Karimi-Abdolrezaee,Caitlin E. Hill, Joseph S. Sparling, Jason R. Plemel, Ward T. Plunet,Eve C. Tsai, Darryl Baptiste, Laura J. Smithson,Michael D. Kawaja, Michael G. Fehlings,Brian K. Kwon. A Systematic Review of Cellular Transplantation Therapies for Spinal Cord Injury, J Neurotrauma. 2011 Aug; 28(8): 1611–1682
19. Xu XM, Guenard V, Kleitman N, et al: Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. J Comp Neurol 351:145-160, 1995
20. Boyd JG, Doucette R, Kawaja MD: Defining the role of olfactory ensheathing cells in facilitating axon remyelination following damage to the spinal cord. FASEB J 19:694-703, 2005
21. Richter MW, Roskams AJ: Olfactory ensheathing cell transplantation following spinal cord injury: hype or hope?. Exp Neurol 209:353-367, 2008
22. Nistor GI, Totoiu MO, Haque N, et al: Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. Glia 49:385-396, 2005
23. Keirstead HS, Nistor G, Bernal G, et al: Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 25:4694-4705, 2005
24. Angelo C, Lepore Ajai, Bakshi, Sharon A et al. Neural precursor cells can be delivered into the injured cervical spinal cord by intratechal injection at the lumbar cord. Brain Research. 2005; 1045:206-216.
25. Karimi-Abdolrezaee S, Eftekharpour E, Wang J at al. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci.2006;26(13): 3377-3389.
26. Liang P, Jin LH, Liang T et al. Human neural stem cells promote corticospinal axons regeneration and synapse reformation in injured spinal cord of rats. Chin Med J(Enlg).2006;119:1331-1338
27. Westerlund U, Svensson M, Moe MC, et al: Endoscopically harvested stem cells: a putative method in future autotransplantation. Neurosurgery 57:779-784, 2005
28. Phinney DG, Prockop DJ: Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views. Stem Cells 25:2896-2902, 2007
29. Parr AM, Tator CH, Keating A: Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant 40:609-619, 2007
30. Lee OK, Kuo TK, Chen WM. Isolation f multipotent mesenchymal stem cells from umbilical cord blood flow. Blood.2004;103(5):1669.