

Spinal cord regeneration

Gourishankar Patnaik MS (Orth), FAOI (USA)

Department of Orthopedic Surgery and Trauma, Melaka Manipal Medical College, Malaysia

Abstract: Research in spinal cord regeneration is catching attention of clinicians and basic scientists. It would almost revolutionise the life and disease outcome of these unfortunate patients if we can work out a cost effective and practical treatment regimen for these victims who are unfortunately in the prime of their productive years. It was gratifying to learn that nerves in peripheral nervous system (PNS), which are outside the brain or spinal cord, did regrow. It is exciting to learn that the prospects of regrowth of spinal cord improves when these PNS cells are implanted in damaged spinal cord. Spinal cord injury is a global epidemic. A lot of research is going on in this field. Axonal regeneration, electric stimulation, Netrins, stem cells etc are few exciting fields in the area of research. It is ongoing research whereby the ability to grow human motor neurons in the laboratory will provide new insights into disease processes and could be used as alternative to animal models for finding therapeutic targets and testing drugs.

Keywords: axonal regeneration, spinal cord regeneration, spinal cord Injury, trauma

INTRODUCTION

“A disease not to be treated” was how an Egyptian papyrus referred to spinal cord injury more than 3,500 years ago¹. When this first medical document to describe spinal cord trauma was translated into English in 1930, medical textbooks around the world still reiterated it that spinal cord injuries were untreated and even today after the passing of several millennia, physicians are still unable to treat injuries to the spinal cord. Only in the middle of the twentieth century did medicine learn to keep spinally injured people alive. The resulting paralysis, however, was considered irreversible. Dead nerve cells in the spinal cord, scientists believed, could not be replaced; severed nerve fibres would never regenerate; paralysed people would never walk again. With hardly any research in this area, there was no progress, which in turn reinforced the impression that the problem was intractable².

Research to find a cure for spinal cord injury (SCI) may seem like a recent event. Yet, scientist Theodor Schwann reported as early as 1830 that nerve cells in rabbits showed signs of regrowth or regeneration. Santiago Ramon Cajal later described, in 1890, how damaged nerves in mammals try to regenerate. The problem he found was that they lacked the ability to

make proper connections. Cajal also knew that nerves in the peripheral nervous system (PNS), which are outside the brain or spinal cord, did regrow. He believed that if PNS cells were implanted into the damaged area of the spinal cord, which is part of the central nervous system (CNS) that they could make the injured nerves re-grow. Later, in the early 1900's, scientist J.F. Tello showed nerves could regenerate but need nourishment. Human SCI research did not make any major progress until after World War II. That is when the discovery of antibiotics and improved surgical and critical care techniques helped more individuals survive their initial SCI. Researchers then turned their focus to explore the spinal cord, its cells and how the nerves work.

During the 1960's, Rita Levi-Montalcini and Viktor Hamburger discovered a substance that nourished nerve cells to help them grow. This was the first nerve growth factor (NGF). Many new growth factors were discovered during the next 20 years, such as brain derived neurotrophic factor (BDNF) by Yves Barde. Scientists also learned that different cells respond to different growth factors. Findings by Raisman in 1969 showed that nerves can make new connections and that the central nervous system has the capacity to reorganize. By the 1980's, scientists had success using peripheral nerve grafts in rats to show that axons can regrow. However, Martin Schwab showed that blockers or inhibitors found in the CNS stopped this growth. Researchers' next focus was to find ways to prevent these blockers from stopping nerve fibers' growth. Increased funding during the past 15 years has provided for more research in the area of spinal cord injury. The ultimate goal is for an individual

Address for Correspondence :

Prof Dr Gourishankar Patnaik
Head, Dept of Orthopaedics and Trauma,
Melaka Manipal Medical College, Jalan Batu Hampar,
Bukit Baru 75150, Melaka Malaysia
Tel + 60176350147, E-mail: drgsp66@yahoo.com

to regain full function. However, “cure research” technically involves all phases of care, beginning with the effective handling of the patients at the time of injury, during acute care and through rehabilitation³.

The new hope touches the lives of millions of people. Spinal cord injury (SCI) is a global epidemic. Based on conservative average annual incidence of 22 people / million population in the western and developing world³ it is estimated that over 130,000 people each year survive a traumatic spinal cord injury and begin a “new and different life” bound to a wheelchair for 40 years or more. With an average age at injury of 33.4 years and most injuries occurring at the age of 19⁴ and life expectancy diminished only by an average less than 10 %, and advances in health maintenance and emergency healthcare, it is clear that the population of people living with spinal cord injuries is steadily increasing around the world. By 2005, new injuries will swell the total world population of people living with spinal cord injury induced paralysis to over 2.5 million.

The economic impact on the community, in terms of the long-term cost of care and cost of social welfare support reaches in excess of tens of billions of dollars each year. Reliable reports have estimated the cost in the United States alone at \$ 7.7 billion dollars annually. In Canada that figure is \$1.5 billion, over \$500 million British Pounds in the United Kingdom and Australia around \$1 billion^{5,6}.

NEWER RESEARCH

A lot of research is now ongoing in this field. It is doubtful that a single researched element will provide the ultimate cure for spinal cord regeneration, but what will really happen is that shared evidence from many research trials may point the way toward figuring out ultimately what needs to be done. This will still take a while, although optimism exists but unless miraculous events occur, chances are that people will still need to wait, and potentially a cure may not exist for people whose injuries have occurred some time ago. Hope remains though that one day, people with paralysis will be able to regain at least some of their lost abilities: breathing, sexual function, bladder and bowel control, walking. Implications for other diseases of the nerves, particularly such brain disorders as stroke and Alzheimer's and Parkinson's diseases can also be great, as spinal cord and brain research are closely intertwined⁷.

One of the present areas of research is in axon regeneration. In 1988, Martin Schwab discovered two myelin-associated proteins that inhibit growth in the damaged mammalian spinal cord, a revolutionary finding. Until then, it was believed that the cord's inability to regenerate was due only to the absence of nerve growth factors. He has followed up his research with many published articles including this one published in 2009 on the differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. Nogo-A is the myelin associated neurite growth inhibitory protein and lesioned rats treated with antibodies against Nogo-A, showed increased regeneration, neuronal reorganization and behavioural improvements⁸. His research also induced nerve regeneration in the rat spinal cord by blocking damaging proteins with an antibody called IN-1. With this treatment, regenerating axons grow about 11 millimetres; without treatment, they do not grow even one millimeter.⁹ He also reported dramatic regrowth of nerves in partially severed rat spinal cords after treatment with a combination of the antibody IN-1 and the growth-promoting factor NT-3¹⁰.

Damaged nerves are also made to grow for regeneration to occur. The neurotrophic proteins function as “growth factors and help prevent cell death. They also work like a “nerve fertilizer” to help neurons survive and nerves regenerate, allowing messages to flow up and down the spinal cord again. Scientists are studying several growth factors and how they can be used in treating spinal cord injury. Each growth factor has very specific target cells that it works on. They include NT-3 (Neurotrophin 3); BDNF (brain derived neurotrophic factor); aFGF (acidic Fibroblast Growth Factor) and NGF (nerve growth factor)¹¹⁻¹³.

Dr Ida Black and his team stunned the research field in the year 2000 when they succeeded in converting stem cells derived from the bone marrow of humans and rats into neuron-like cells for potential transplantation to treat a variety of neurological diseases¹⁴. Growth factors such as BDNF and NT-3 are being used along with neurons ‘grown’ in the lab from bone marrow stem cells to promote regrowth of spinal cord nerve fibers. Researchers are analysing their use in spinal cord injured rats to define how they may improve nerve growth.

Another component of nerve regeneration, which researchers are working on, is with different substances to guide nerve growth so nerves grow past the injury site

and reconnect with the proper nerve. Netrins are proteins produced in the brainstem that “attract” nerve cells. They encourage nerve cells to migrate to and grow branches toward a “target.” Dr. Mark Tessier-Lavigne of Stanford University has identified netrins in several animal models and is evaluating their use with spinal cord injury¹⁵. Neural glues are substances that can fuse together the ends of damaged nerve axons. Scientists at the Centre for Paralysis Research, Purdue University, used polyethylene glycol (PEG) in guinea pigs, which helped to partially restore nerve function immediately following spinal cord compression injury. It is thought that this helps restore nerve cell membranes disrupted by the spinal cord injury¹⁶. Fibroblast cells, commonly found in the skin, and act as a “bridge” across a spinal cord lesion. Scientists genetically engineer these fibroblast cells to produce neurotrophin-3. The cells can then stimulate regrowth. Rats showed improved leg function following implantation of these fibroblasts in research done by Dr. Marion Murray at MCP Hahneman University¹⁷.

Electrical stimulation is also used as a method of nerve regeneration but it is still in the human clinical trials. Researchers at Purdue University’s Centre for Paralysis Research and Indiana University School of Medicine are using low-level electrical stimulation on paralysed dogs. They implant a small battery pack, known as an extra spinal oscillating field stimulator (OFS), near the dog’s spine. It sends a weak electrical signal (thousandths of a volt) to the site of injury. This helps regenerate cells and guide growth in the damaged nerves. In about a third of the cases, the dogs improved significantly¹⁸.

Other key cure research focus areas are that of neuroprotection, which includes methylprednisolone, interleukin-10, GM-1 Ganglioside (Sygen), Glutamate (AMPA) Receptor Blockers and 4-Aminopyridine. In 1990, the first effective treatment for acute SCI was identified. Clinical Trials show that neurological recovery in Human spinal cord injuries improves by an average 20% if large doses of the steroid methylprednisolone (MP) are administered within eight hours of injury. It was the first drug shown to reduce spinal cord damage in humans by preventing swelling and inflammation at the injury site. It is now the American “standard of care”¹⁹. Interleukin-10 (IL-10) is a potent anti-inflammatory substance. Researchers at the Miami Project, led by John Bethea, PhD, are using IL-10 in their research with rats and rats treated with IL-10 recovered significant use of their hind limbs during the

weeks following injury. However, further study is needed before this drug can be tested on humans²⁰. The drug Fampridine-SR (also known as 4-aminopyridine or 4-AP) does not have neuroprotective effects. However, research shows that 4-AP allows nerve signals to pass along axons which have lost their “insulation wrapping” due to injury. These early trials show that in some chronically paralysed patients, 4-AP can increase the ability of axons to conduct signals and thus restore some lost function after injury. Future studies are still being done to determine its efficacy²¹.

STEM CELLS

In another major development, Fred Gage reported in 1994 that skin cells, genetically engineered to secrete growth factors and neurotransmitters, cause massive regeneration of sensory nerve cells in the spinal cord. Genetically engineered cells with growth factors believed to cause regeneration of movement controlling cells are now being tested²². In 1996, Lars Olson of the Karolinska Institute reported for the first time having achieved “true functional recovery” of a severed adult rat spinal cord. Olson and his colleagues used a five-step strategy, including implanted peripheral nerve bridges stabilized by using fibrin glue mixed with fibroblast growth factor. It should be noted that the procedure was successfully done on only a few animals and none recovered the ability to walk. These experiments will have to be replicated in other laboratories around the world to confirm the findings²³.

As much as it’s important to understand how to repair something, it may also be necessary to understand why it won’t work. In the late 2000s, one study that may be used medically in future evaluates the blood clotting protein fibrinogen. It was found in people with damaged spinal cords that this protein was present in highly excessive amounts, and that it may be inhibiting the repair of neurons. There are ways to block the protein’s action and these might be indicated in future treatment²⁴.

One of the most recent breakthroughs has been in 2006 in a research project published in the Journal of Neuroscience, the Drexel University College of Medicine in Philadelphia led by Professor of Neurobiology and Anatomy John Houle has observed in Laboratory experiments how a nerve taken from a lab animal and transplanted across spinal cord injury combined with an enzyme digestion of scar material can lead to a regeneration of the injured nerve tissue and recovery of

limb movements. The milestone of this lab demonstration is that the process is equally applicable to animals that are newly injured as well as in animals with long-term injuries because of the ability to use the implanted nerve bridge to direct regeneration towards a specific target area in the spinal cord. The next follow up of this experiment will be to test the ability of that specific enzyme, chondroitinase, to modify scar tissue, reducing its normal inhibitory nature and facilitating growth beyond the implanted nerve bridge. Dr. Houle: "This study represents a major milestone in the battle to return spinal cord injury patients to a state of mobility, however there is still a lot of work to be done to adapt this procedure to human use"²⁵.

A lot of research is still ongoing in the field of spinal cord injury and regeneration. For many years it was assumed that spinal cord regeneration was not possible. Paralysis, often resulting from damaged spinal cords, was likely to be permanent, and many peoples' lives were forever altered by a spinal cord injury. This is still the case today, but what has changed is the degree of optimism many people hold about someday being able to use medical techniques to fix spinal cord injuries and restart the damaged nerves that have lost function after an injury has occurred. A recent study conducted at the Korea University Medical Centre suggests that implants of exogenous neural stem cells may promote regeneration in aging organisms through stimulation of endogenous neurogenesis²⁶.

Spinal cord injury (SCI) in adults often leads to permanent functional deficits because the regeneration of injured axon and the reorganization of the remaining circuitry are insufficient in the human central nervous system. Therefore promoting axonal regeneration is one of the essential goals to be achieved for effective repair from SCI. Wnt ligands are a family of glycoproteins that have diverse and essential roles in the development, cell growth and human diseases. Experimental work done in rats suggested the neurological recovery after SCI in rats were improved by Wnt secreting fibroblasts which were injected intramedullarily at 1 week after SCI. ME – MRI shows that axonal regeneration was better in Wnt group through the injured site in spinal cord²⁷.

Cell transplantation therapies have been developed experimentally for some CNS disorders. It also came to be realised that adult mammalian CNS has some regenerative capacity. Thus regeneration of the damaged CNS is becoming feasible from the clinical aspect,

although it still remains primitive. Therefore it is obvious that both 1) activation of the endogenous regenerative capacity and 2) cell replantation therapy are very important strategies for CNS repair. Elucidation of the molecular and cellular mechanisms of the stem cell regulation and normal CNS developmental process in combination with molecular – targeted drug discovery would be essential for future development of innovative therapeutic interventions of various CNS damages including SCI, stroke and neurodegenerative disorders²⁸.

CONCLUSION

Experimental spinal cord injury is no longer incurable. Many papers have appeared over the past few years reporting functional recovery following a variety of treatments. These have included interventions that affect myelin inhibitory molecules and their receptors, or inhibitory chondroitin sulphate proteoglycans, and treatments in which the regenerative potential of axons has been stimulated through growth-factor receptors or manipulation of internal signalling pathways. Researchers have found that laboratory grown motor neurons may someday provide cells for transplantation into individuals with spinal injuries and motor neurone related diseases such as amotrophic lateral sclerosis as the stem cells develop into motor neurons, which transmit messages from central nervous system to muscles. Also of interest is to observe the role of electric stimulation, axonal regeneration and role of neuroprotective agents. Specific research goals include improving neuronal survival, promoting functional recovery through axonal regeneration, compensating for myelination, and replacing lost cells. The continued basic research on the properties of these cells and development of appropriate animal models of repair will pave the way for successful clinical application.

REFERENCES

1. Edwin Smith *papyrus*. 1550 B.C.E. Rare Book Room of New York Academy of Medicine.
2. Luba Vikhanski. Thinking the Unthinkable About Spinal Cord Regeneration. In *Search of the Lost Cord: Solving the Mystery of Spinal Cord Regeneration*. Washington: Joseph Henry Press, 2001.
3. Spinal Cord Injury Information Network Online. Research for the Cure in Spinal Cord Injury: History of Cure Research. <http://www.spinalcord.uab.edu/show.asp?durki=47987>.
4. United Nations Population Division. Total world population report. Geneva: WHO 2009.

5. National Spinal Cord Injury Association. Report on injuries in the United States. Washington: NSCIA 2009.
6. International Campaign for Cures of Spinal Cord Injury Paralysis Online. General Information. http://www.campaignforcure.org/iccp/index.php?option=com_content&task=view&id=13&Itemid=28 Wisegeek. Is spinal cord regeneration possible? <http://www.wisegeek.com/is-spinal-cord-regeneration-possible.htm>
7. Maier IC, Ichiyama RM, Courtine G, et al. Differential effects of anti-Nogo-A-antibody treatment and treadmill training in rats with incomplete spinal cord injury. *Brain* 2009; 132: 1426-40.
8. Schwab ME, Bartholdi D. Degeneration and regeneration of axons in the lesioned spinal cord. *Physiology Review* 1996; 76:319-70.
9. Raineteau O, Fouad K, Noth P, Thallmar M, Schwab ME. Functional switch between motor tracts in the presence of the mAb IN-1 in the adult rat. *Proc National Academy Science USA* 2001; 98(n): 6929-34.
10. Maisonnier PC, Le Beau MM, Espinosa R 3rd, et al. Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations. *Genomics* 1991; 10: 558-68.
11. Tuszynski M., Blesch A. Nerve growth factor: from animal models of cholinergic neuronal degeneration to gene therapy in Alzheimer's disease. *Brain* 2001; 146:79-123.
12. Sun W, Sun C, Lin H, Zhao H, Wang J, Ma H et al. The effect of collagen-binding NGF-beta on the promotion of sciatic nerve regeneration in a rat sciatic nerve crush injury model. *Biomaterials* 2009; 27: 4649-56.
13. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* 2000; 61: 364-70.
14. Marcos S, Backer S, Causeret F, Tessier-Lavigne M, Bloch-Gallengo E. Differential roles of Netrin-1 and its receptor DCC in inferior olivary neuron migration. *Mol Cell Neurosci* 2009; 41: 429-39.
15. Borgens RB, Shi RY. Immediate recovery from spinal cord injury through molecular repair of nerve membranes with polyethylene glycol. *FASEB J* 2000; 14: 27-35.
16. Miya D, Tessler A, Giszter S, Mori F, Murray M. Fetal transplants alter the development of function after spinal cord transection in newborn rats. *J Neurosci* 1997; 17:4856-72.
17. Borgens R, Toombs J, Breur G, et al. An imposed oscillating electrical field improves the recovery of function in neurologically complete paraplegic dogs. *J Neurotrauma* 1999; 16: 639-57.
18. Bracken MB, Shephard MJ, Holford TR, et al. Methylprednisolone or trilasol mesylate administration after acute spinal cord injury: 1-year follow up. Results of the third National Acute Spinal Cord Injury randomised controlled trial. *JAMA* 1997; 277: 1597-604.
19. Brewer KL, Bethea JR, Yeziers RP. Neuroprotective Effects of Interleukin-10 Following Excitotoxic Spinal Cord Injury. *Experimental Neurology* 1999; 159: 484-93.
20. DeForge D, Nymark J, Lemaire E, et al. Effect of 4-aminopyridine on gait in ambulatory spinal cord injuries: a double-blind, placebo-controlled, crossover trial. *Spinal Cord* 2004; 42: 674-85.
21. Tuszynski MH, Senut MC, Ray J, Roberts J, U H.-S., Gage FH. Somatic gene transfer to the adult primate CNS: In vitro and In vivo characterization of cells genetically modified to secrete nerve growth factor. *Neurobiol Dis* 1994; 1:67-78.
22. Lars Olsen, Henrich Cheng, Yihai Cao. Steps towards healing damaged spines. *Science News* 1996; 150:52-53.
23. Adams RA, Bauer J, Flick MJ, Sikorski SL, Nuriel T, Lansman H et al. The fibrin-derived $\gamma^{377-395}$ peptide inhibits microglia activation and suppresses relapsing paralysis in central nervous system autoimmune disease. *The Journal of Experimental Medicine* 2002; 204: 571-82.
24. Houle JD, Tom VJ, Mayes D, Wagoner G, Phillips N, and Silver J. Combining an autologous peripheral nervous system "bridge" and matrix modification by chondroitinase allows robust, functional regeneration beyond a hemi section lesion of the adult rat spinal cord. *J Neurosci* 2006; 26: 7405-15.
25. Dong-Hyuk Park, David J Eve, Paul R Sanberg, et al. Induction of neuronal proliferation in the dentate gyrus of aged rats by grafted human neural stem cells. *Proceedings of 2nd international Congress of Asia Oceania Neurotrauma Society* 2010.
26. Sang Ryong Jeon, Hyung Il Seo Joong Kee Min, Seong Who Kim. Therapeutic effects of Wnt secreting fibroblast implantation in spinal cord injury of rat. *Proceedings of 2nd international Congress of Asia Oceania Neurotrauma Society* 2010.
27. Okano H, Sawamoto K. Neural Stem Cells: involvement in adult neurogenesis and CNS repair. *Phil Trans R Soc B* 2008; 363,2111-22.

ACKNOWLEDGEMENTS

The author wishes to thank Ms Michelle Lee Hui Lim a final year medical student for her contribution.