Introduction Part B

Bone Marrow Stromal Cells for Repair of the Injured Spinal Cord

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INTRODUCTION

In 1927 Harvey Cushing described the outcome for soldiers with spinal cord injury (SCI) sustained during World War I: "Fully 80 percent died in the first few weeks in consequence of infection from bedsores and catheterization. Only those cases survived in which the spinal cord lesion was a partial one" [1]. Nowadays, this has been reversed. In well-organized systems of care for trauma and SCI and due to improved critical-care medicine most patients survive the initial hospitalization. At present, there is no treatment available that effectively re-establishes disrupted axonal circuitries that are necessary to restore injury-induced functional deficits. Due to the lack of a cure and the improved health care, the number of wheelchair bound people increases steadily each year. Currently, in the United States there are an estimated 400,000 people with SCI, with an annual incidence of 11,000 (The National Spinal Cord Injury Statistical Center, Birmingham, AL). In Western European countries similar leading causes of SCI are obtained as in the United States, with vehicular crashes and falls as leading causes of SCI and predominantly young males affected[2,3]. In contrast to the developed countries, in the less developed countries a shift of etiology can be observed towards falls[4] and violence[5-7].

Following the first medical care in a hospital, continuing medical care is necessary to maintain the SCI patient's health and quality of life. This does not lead to functional repair. Repair-promoting pharmaceutical and/or surgical interventions will be necessary to significantly change the functional outcome after SCI. Transplantation of repair-supporting cells is considered a candidate repair approach. A bone marrow stromal cell (BMSC) transplant has shown great promise for spinal cord repair. This chapter will give an overview of the pathophysiology, clinical consequences, assessments, and treatments of SCI and will then focus on BMSC as a possible therapy for SCI. In addition, the SCI model system used in subsequent chapters will be explained.

PATHOPHYSIOLOGY AND CLINICAL CONSEQENCES

A direct force to the vertebral column can cause damage to bony and soft tissue structures. Torn ligaments or fractures can cause instability of the vertebral column with

potential risk of additional damage. Fracture dislocation and hematomas can directly compress the spinal cord and cause immediate neural cell death, axon damage and demyelination, resulting in instant loss of motor and sensory function. After the first destructive events, a sequence of molecular and cellular pathophysiological events, including an aggressive inflammatory response within the damaged tissue, leads to additional tissue loss at the injury epicenter and at distant sites (secondary injury)[8,9]. The functional consequences of SCI are highly variable and depend on the degree of tissue damage, which in turn depends on the impact severity. In patients with SCI with a relatively small amount of tissue damage, some endogenous recovery of function can be observed, which is most likely resulting from plasticity of the spinal nervous tissue[10,11]. In people with SCI with extensive tissue damage the neurological deficits are generally major and permanent. There are very few reports of people with a large injury that regain motor function to a degree that independence can be achieved.

Over 95% of SCI patients survive their initial hospitalization. The relatively young age when SCI occurs, improved medical care, and lack of effective therapies are responsible for the continually increasing number of paralyzed people with SCI. This puts a high financial burden on the patient, his/her family, and society[3,12]. The psychological consequences of SCI should not be underestimated and appropriate guidance of patient and family should have an important place in the management of SCI[13-15]. Patients need time to accept their deficits. One can expect an initial period of denial and/or inability to fully comprehend the consequences of the paralysis caused by the injury. After the patient realizes his/her fate to the fullest extent, a period of acceptance will have to run its course[13]. After that, the patient needs to learn to live with his/her disabilities, and this may be accompanied by bouts of depression. The mental state of the patient can have its effect on his/her medical treatments[14].

SCI is the second most expensive condition to treat in the United States after respiratory distress syndrome in infants and is ranked third in medical conditions requiring the longest stay in hospitals[16]. The costs of lifetime care for a SCI patient varies between 1 and 3

million dollars. The Center for Disease Control in the United States estimated that about 10 billion dollars are spent yearly on SCI treatment excluding the management of pressure ulcers, a common adverse effect of SCI, which adds another billion dollars per year[17].

TREATMENT

An acute and a chronic phase can be distinguished after SCI. Since SCI is often a consequence of severe accidents, initial treatment is generally focused on stabilization of the patient. There is insufficient evidence that would support standards of care during the acute phase of SCI. It is advised to maintain patients in an intensive care unit for close monitoring of respiratory and hemodynamic complications. For adequate spinal perfusion, which can be at risk due to injury-induced edema, a mean arterial pressure of 85-90 mmHg should be maintained[18]. Depending on the type of injury, surgical interventions should be considered to decompress the spinal cord and or stabilize the spinal column[19,20]. Decompression surgeries may accelerate functional improvements and result in shorter hospitalization and rehabilitation periods[17,21] . However, it does not result in an improved functional outcome[22]. A lack of consensus of care during the acute phase of SCI is in part due to the large variability among injuries and makes its early management complicated. If bone fragments continue to compress the spinal cord, early surgery may be vital to prevent exacerbation of spinal cord tissue destruction. However, in cases without a clear sign of such urgency there is no consensus on whether and what type of early surgical/clinical interventions must be implemented. The type of surgical intervention should be considered on a case-to-case basis, which makes it complicated to study the efficacy of intervention in the acute phase after SCI in randomized and controlled clinical trials.

Besides surgical interventions, pharmacological treatments to limit the secondary injury after SCI are often considered. The best-known treatment is a high dose of the glucocorticosteroid, methylprednisolone sodium succinate (MPSS) within 8 hours after the injury[23-25]. Experimentally it was demonstrated that a high dose of MPSS reduces the inflammatory response and limit tissue loss after damage to the spinal cord. The effects of

MPSS in patients with SCI were investigated in 3 consecutive National Acute Spinal Cord Injury Studies (NASCIS)[23-25]. The results demonstrated that MPSS treatment in the acute phase of SCI resulted in neurological improvements up to 6 months after injury. After a thorough review of the results from the NASCIS studies and a more comprehensive assessment of the benefits and risks involved in high dose MPSS treatment, the therapeutic benefits are now disputed[26-28]. Especially in patients with complete SCI high dose steroid treatment can lead to adverse effects such as myopathy and wound infection that may negatively influence functional outcome and in some cases may be life-threatening[28,29]. Currently, many SCI clinics worldwide have discontinued the 'standard' acute administration of MPSS after SCI.

Treatment paradigms in the chronic stage after SCI are multidisciplinary and intensive. Different complications may occur that each demands specific interventions. For instance, SCI can lead to pain[14,15], decreased fertility[30], and autonomic dysreflexia with loss of bladder and bowel control[31]. It has to be taken into consideration that many SCI patients get accustomed to the specific injury-related pain they experience and as a result reveal their distress to their physician often at a late stage[32,33]. For some SCI-related conditions, such as decreased fertility, it is the patient's personal desire that should guide the physician's actions. Other common problems that arise after SCI are septicemia, respiratory insufficiency, and pneumonia due to muscle atrophy. These complications may cause clinical deterioration and could eventually result in death. They often occur without typical symptoms. It is imperative that SCI patients receive annual screenings and long-term follow-ups to prevent these secondary complications. It is advised to treat patients on a regular basis with pneumococcal and influenza vaccine to prevent opportunistic infections. Monitoring the skin and urinary tract and implementing aggressive treatments against pressure ulcers and urinary tract infections is needed to reduce the risk of septicemia. Appropriate nutrition and exercise should also be incorporated in the (new) lifestyle. Rehabilitation programs should be implemented to reduce the risk of cardiovascular disease[34].

BONE MARROW STROMAL CELL THERAPY

Mesenchymal stem cells from bone marrow (here referred to as bone marrow stromal cells (BMSCs)) have therapeutic potential for the injured spinal cord[35]. BMSC were shown to differentiate into bone, fat, tendon and cartilage cells[36]. Although still debated, it has been reported that BMSC can transdifferentiate in vitro into liver cells[37], skeletal cells[38], cardiac muscle cells[39], and neural cells[37,40]. Besides this ability, BMSC are also known to produce different types of growth factors that could potentially influence nervous tissue repair positively. Together, these abilities make BMSC interesting for repair strategies for the injured spinal cord.

Several other aspects make BMSC interesting candidates for cell-based approaches for central nervous system repair. Firstly, BMSC are relatively easy to obtain from a fairly routine bone marrow extraction followed by a quick centrifuge and culture procedure to remove the hematopoietic cells. Secondly, BMSC are easy to culture as they do not need complicated growth media or special culture circumstances. Basic cell culture equipment is sufficient to successfully culture millions of BMSC. Thirdly, BMSC are easy to transduce with viral vectors which, if necessary, may be helpful to boost the overall reparative abilities of the cells. The use of viral vectors to genetically modify cells prior to transplantation has not yet become mainstream as there are some biological and ethical issues that need to be resolved. Finally, BMSC do not have the ethical concerns that embryonic or fetal stem cells have, and therefore circumvent public rejection as a possible treatment for neural and non-neural trauma and disorders.

At this time, there is no irrefutable evidence that BMSC transplanted into the damaged nervous tissue differentiate into neural cells that successfully replace lost cells. Also, there is no convincing evidence that neural cells derived from grafted BMSC contributed to functional improvements after transplantation. As long as the potential of BMSC for differentiation into neural cells is in debate, the ability to produce and secrete different types of growth-promoting molecules, which include several neurotrophins and cytokines, is the more interesting and more likely characteristic of BMSC that makes these cells

important candidates for spinal cord repair approaches. By releasing these molecules, BMSC can positively influence the consequences of spinal cord injury and support anatomical and functional repair (Figure 1).

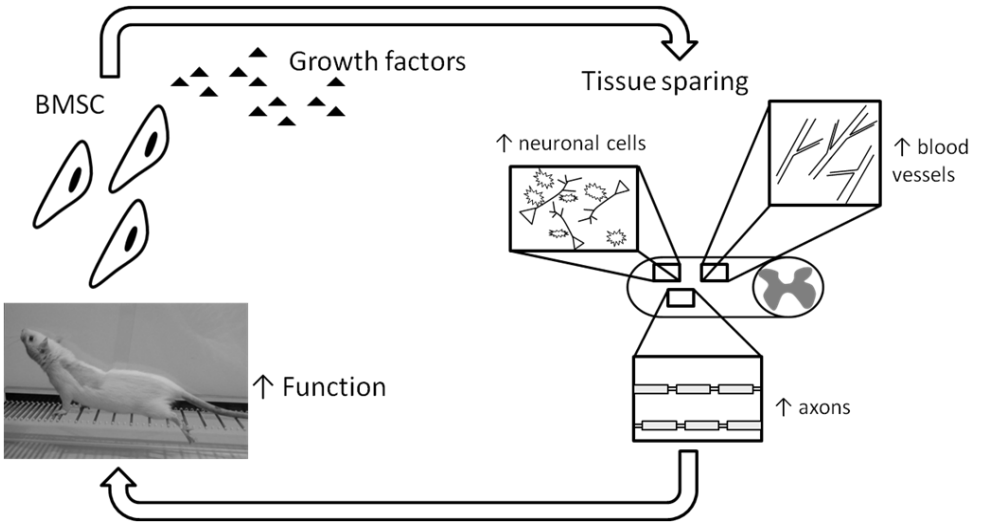

Fig. 1. BMSC secrete various growth factors, including BDNF, VEGF, NGF and NT-3. These factors are thought to limit the loss of tissue in the injured spinal cord, contributing to the increased functional outcomes after BMSC transplantation.

RAT MODEL SYSTEM

Promising therapies for spinal cord injury are typically tested in rodent models, and mostly in rats. Similar as in humans, a SCI in the rat results in progressive loss of the grey and white matter creating large fluid filled cysts. Proliferation and activation of astrocytes result in formation of scar tissue, which acts as a barrier for axonal regeneration. Importantly, as in humans, there is no spontaneous regeneration in the injured spinal cord in rodents. The histological similarity between human and rat spinal cord injury has made the rat an extensively studied model for experimental therapeutic strategies, including BMSC transplantation.

The most widely used model of spinal cord injury involves a spinal cord contusion inflicted by an impactor device. A contusion is clinically the most frequently occurring type of spinal

cord injury; approximately 75% of all human injuries are contusions. The consequences of a contusive injury in rats are similar as the known consequences in the contused human spinal cord. Figure 1 shows the rat model system for spinal cord contusive injury.

An alternative model for a contusion-like spinal cord injury is the clip compression model. The main difference between the impactor-inflicted contusion and the clip-inflicted compression is time. With an impactor the spinal cord is compressed for a brief moment of time while with a clip the spinal cord is compressed for a longer, regulatable, time. The clip model is clinically more relevant as most spinal cord injuries are inflicted by a lasting compression rather than a brief one.

There are a number of other, non-contusive, spinal cord injury models employed in laboratories around the world to test treatment paradigms. These are valuable in their own right to investigate the underlying mechanisms and/or validity of certain approaches. Partial transections of specific regions in the spinal cord are used especially to study the effects of treatments that aim to promote axonal regeneration; specific descending or ascending pathways can be damaged with relatively small local knife cuts and the regeneration response quantified at later time points. The involvement of specific axonal pathways in locomotor function can also be investigated using partial transections. The main disadvantages of partial transections are the low clinical relevance and the possible misinterpretation of results due to compensatory sprouting, i.e., other previously non-involved axonal pathways become involved in particular functions. Another model that has been used is the complete transection of the spinal cord. Although this is not often seen in the clinic, complete transections are particularly advantageous to study cell types for their ability to promote regeneration of damaged axons without contaminating sprouting of undamaged pathways and to serve as bridging material between spinal cord stumps. This model is also suitable to study the efficacy of synthetic or natural biomaterials for their efficacy to serve as carrier of cells or drugs. A disadvantage besides the low clinical relevance is that rats with a completely transected spinal cord are more laborious to maintain.

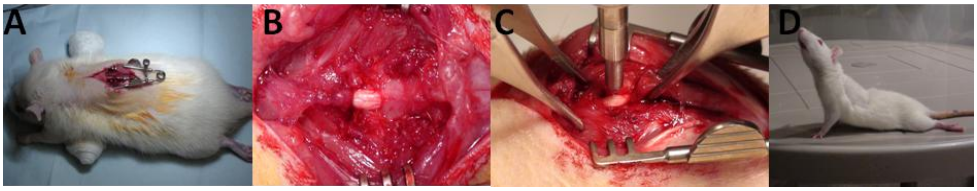

Fig. 2. Rat spinal cord contusion model. A. A laminectomy is performed exposing the underlying spinal cord. B. Enlarged view of the exposed spinal cord segment. C. A computerized impactor is used to contuse the spinal cord. The piston is attached to a sensor to record velocity, force and displacement to ensure consistency. D. A moderate contusion results in loss of function at and below the level of injury and loss of bladder function.

BMSC INJECTION

It is difficult to provide standard guidelines for cell preparation because every cell type requires special conditions and circumstances for optimal isolation and culturing. Cell injection procedures may vary but are essentially similar. The standard procedures to harvest, culture and genetically modify BMSC with lentiviral vectors encoding for green fluorescent protein (GFP) to enable easy identification in vivo, as well as to inject BMSC as used in our laboratory are depicted in Figure 5. The length of the culture (preparation) time for BMSC depends on how many cells are needed to fill the damaged area. Thus, the number of BMSC necessary depends on the overall loss of tissue which, in turn, depends on the severity of the initial insult and on the time between insult and transplantation. Imaging techniques may provide the necessary information to guide the decisions on damaged tissue volumes and number of cells.

There are a number of studies that have explored injection paradigms other than straight acute injections into the injury site. BMSC have been infused systemically or into the 4th ventricle[41], or transplanted acutely into the cervical[42] or thoracic spinal cord[43,44] or into the chronically injured cord[45].

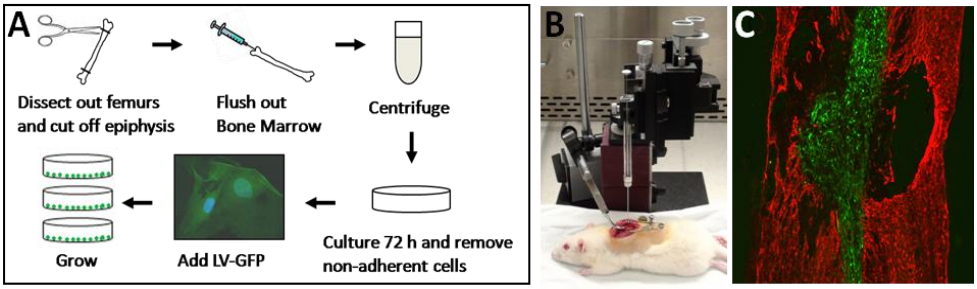

Fig. 3. Transplantation of BMSC. A. BMSC are isolated from femurs of rats by cutting off the epiphyses and flushing out the bone marrow. Cells are plated onto plastic culture dishes. Non-adherent hematopoietic stem cells are removed and the plastic-adherent BMSC are infected with LV-GFP. B. Cells are injected into the spinal cord contusion epicenter using a Hamilton syringe with a pulled glass needle attached, held within a micromanipulator. C. Appearance of transplanted BMSC (green) in the contused rat spinal cord seven days post transplantation (20 µm thick section at 2.5 x magnification). The red color represents immunohistochemically stained glial fibrillary acidic protein (GFAP), a commonly used marker for astrocytes.

TIMING OF TRANSPLANTATION

In an experiment by Nandoe Tewarie and colleagues[46] , BMSC were transplanted into a moderately contused adult rat spinal cord at 15 min, and at 3, 7, and 21 day post-injury and BMSC survival was closely assessed both during the transplantation procedure and up to four weeks after transplantation. In addition, the effect of the timing of BMSC transplantation on tissue sparing was determined. BMSC were collected from culture dishes, kept on ice, and passed through a glass pulled needle for injection into the contusion site. This procedure resulted in a majority (67 %) of the BMSC intended to be transplanted being present in the contusion at 15 min after transplantation. Thereafter, BMSC numbers rapidly decreased. The rate at which cell death occurs is different when transplanting acutely or delayed. In an acute transplantation paradigm (15 min post-contusion) and sub-acute transplantation paradigm (3 days post-injury) BMSC survival is better than in a delayed transplantation paradigm (7 days or 21 days post-injury). The percentages of BMSC in the contusion at seven days after transplantation are 32% and 52% for acute and sub-acute transplantation, respectively, and 9% for delayed transplantation. Four weeks after transplantation, almost no BMSC can be found in either paradigm (see figure 4). Interestingly, the presence of BMSC for this short period of time is sufficient to elicit tissue sparing. Acute and subacute transplantation, but not delayed

transplantation results in neuroprotection, and tissue volumes in these paradigms are strongly correlated with the number of BMSC present[46]. These results indicate that timing of BMSC transplantation is important for optimal survival and neuroprotective effect, with acute and subacute transplantation being superior to delayed transplantation. However, because of the clinical relevance of delayed treatment, it seems imperative to find strategies to improve BMSC survival in delayed paradigms.

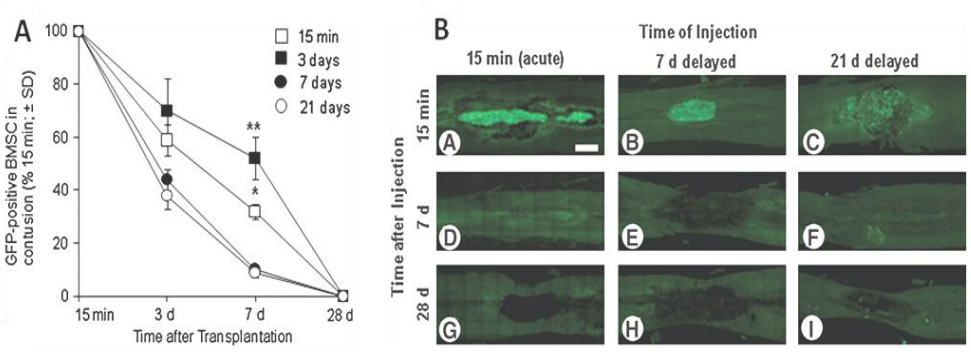

Fig. 4. A. BMSC numbers within a moderate contusion in the adult rat thoracic spinal cord decrease during 28 days post-injection. The rate at which cell death occurs is higher when BMSC are transplanted 7 or 21 days post-contusion, compared to BMSC transplantation 15 min or 3 days after contusion. B. The decreasing transplant is shown at 15 min (A–C), 7 days (D–F), and 28 days (G–I) after an injection at 15 min (acute), 7 days, and 21 days, respectively, post-injury. All microphotographs are from horizontal cryostat sections. (A) Scale bar, 600 mm in A–I.

Previously, using a rat contusion injury model, Hofstetter and colleagues[43] showed that more BMSCs survived when transplanted one week after injury compared to immediately after injury. The surviving cells were located within trabeculae that span the injury site. These data are in disagreement with those from the Nandoe Tewarie study[46] although long-term results were in agreement with only 1% of the cells (about 3000 total) surviving at 4 weeks after grafting.. The difference in early survival between the two studies may be that Hofstetter and co-workers injected the BMSC not only into the contusion but also rostral and caudal thereof into the spinal cord nervous tissue. Possibly, the surviving cells were located nearby but not in the contusion epicenter. Most studies have reported a poor survival of BMSC. Nandoe Tewarie and colleagues[46] demonstrated that the contusion milieu is less detrimental during the first week after injury than the second and fourth

week after injury. What factors are important for BMSC survival *in vivo*? BMSCs are cultured in medium containing 10-20% serum. Factors other than present in serum are not essential for their survival and proliferation within the culture dish. In fact, addition of growth factors such as BDNF, FGF-2, or NT-3 instigates differentiation of the BMSCs into neural-like cells rather than affect survival. To date, the factors that may promote BMSC survival in vivo are unknown and further investigations are necessary to reveal them.

CONCLUSION

Stem cells have gained attraction over the last years in the field of neuroscience. In vitro it has been shown, although still disputed, that Bone Marrow Stromal Cells can transdifferentiate into cells of neural lineage. This has made this adult stem cell type interesting for neural transplantation paradigms. After transplantation of BMSC in the injured spinal cord most cells die. Nevertheless, especially in early transplantation, cells have a neuroprotective effect on the host tissue. This effect may well be the result of secretion of growth factors. Further studies are needed to investigate the true potential of BMSC.

REFERENCES

1. Cushing. Organization and activities of the neurological service American expeditionary forces. . *Ireland MW, ed. The medical department of the United States Army in the world war. Washington, D.C.: Government Printing Office.* 1927:749-758.

2. Ditunno JF, Jr., Formal CS. Chronic spinal cord injury. *N Engl J Med.* Feb 24 1994;330(8):550-556.

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

4. Hoque MF, Grangeon C, Reed K. Spinal cord lesions in Bangladesh: an epidemiological study 1994 - 1995. *Spinal Cord.* Dec 1999;37(12):858-861.

5. da Paz AC, Beraldo PS, Almeida MC, Neves EG, Alves CM, Khan P. Traumatic injury to the spinal cord. Prevalence in Brazilian hospitals. *Paraplegia.* Sep 1992;30(9):636-640.

6. Dincer F, Oflazer A, Beyazova M, Celiker R, Basgoze O, Altioklar K. Traumatic spinal cord injuries in Turkey. *Paraplegia.* Sep 1992;30(9):641-646.

7. Hart C, Williams E. Epidemiology of spinal cord injuries: a reflection of changes in South African society. *Paraplegia.* Nov 1994;32(11):709-714.

8. Blight AR. Macrophages and inflammatory damage in spinal cord injury. *J Neurotrauma.* Mar 1992;9 Suppl 1:S83-91.

9. Hagg T, Oudega M. Degenerative and spontaneous regenerative processes after spinal cord injury. *J Neurotrauma.* Mar-Apr 2006;23(3-4):264-280.

10. Dietz V, Harkema SJ. Locomotor activity in spinal cord-injured persons. *J Appl Physiol.* May 2004;96(5):1954-1960.

11. Dobkin B, Barbeau H, Deforge D, et al. The evolution of walking-related outcomes over the first 12 weeks of rehabilitation for incomplete traumatic spinal cord injury: the multicenter randomized Spinal Cord Injury Locomotor Trial. *Neurorehabil Neural Repair.* Jan-Feb 2007;21(1):25-35.

12. Fiedler IG, Laud PW, Maiman DJ, Apple DF. Economics of managed care in spinal cord injury. *Arch Phys Med Rehabil.* Nov 1999;80(11):1441-1449.

13. Boekamp JR, Overholser JC, Schubert DS. Depression following a spinal cord injury. *Int J Psychiatry Med.* 1996;26(3):329-349.

14. Widerstrom-Noga EG, Felipe-Cuervo E, Broton JG, Duncan RC, Yezierski RP. Perceived difficulty in dealing with consequences of spinal cord injury. *Arch Phys Med Rehabil.* May 1999;80(5):580-586.

15. Widerstrom-Noga EG, Felix ER, Cruz-Almeida Y, Turk DC. Psychosocial subgroups in persons with spinal cord injuries and chronic pain. *Arch Phys Med Rehabil.* Dec 2007;88(12):1628-1635.

16. Winslow C, Bode RK, Felton D, Chen D, Meyer PR, Jr. Impact of respiratory complications on length of stay and hospital costs in acute cervical spine injury. *Chest.* May 2002;121(5):1548-1554.

17. McKinley WO, Jackson AB, Cardenas DD, DeVivo MJ. Long-term medical complications after traumatic spinal cord injury: a regional model systems analysis. *Arch Phys Med Rehabil.* Nov 1999;80(11):1402-1410.

18. Botel U, Glaser E, Niedeggen A. The surgical treatment of acute spinal paralysed patients. *Spinal Cord.* Jul 1997;35(7):420-428.

19. Brodkey JS, Miller CF, Jr., Harmody RM. The syndrome of acute central cervical spinal cord injury revisited. *Surg Neurol.* Oct 1980;14(4):251-257.

20. Fehlings MG, Perrin RG. The timing of surgical intervention in the treatment of spinal cord injury: a systematic review of recent clinical evidence. *Spine (Phila Pa 1976).* May 15 2006;31(11 Suppl):S28-35; discussion S36.

21. Papadopoulos SM, Selden NR, Quint DJ, Patel N, Gillespie B, Grube S. Immediate spinal cord decompression for cervical spinal cord injury: feasibility and outcome. *J Trauma.* Feb 2002;52(2):323-332.

22. Chen TY, Dickman CA, Eleraky M, Sonntag VK. The role of decompression for acute incomplete cervical spinal cord injury in cervical spondylosis. *Spine (Phila Pa 1976).* Nov 15 1998;23(22):2398-2403.

23. Bracken MB. Steroids for acute spinal cord injury. *Cochrane Database Syst Rev.* 2002(3):CD001046.

24. Bracken MB, Shepard MJ, Collins WF, et al. A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Results of the Second National Acute Spinal Cord Injury Study. *N Engl J Med.* May 17 1990;322(20):1405-1411.

25. Bracken MB, Shepard MJ, Holford TR, et al. Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury. Results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial. National Acute Spinal Cord Injury Study. *Jama.* May 28 1997;277(20):1597-1604.

26. Nesathurai S. Steroids and spinal cord injury: revisiting the NASCIS 2 and NASCIS 3 trials. *J Trauma.* Dec 1998;45(6):1088-1093.

27. Nesathurai S. The role of methylprednisolone in acute spinal cord injuries. *J Trauma.* Aug 2001;51(2):421-423.

28. Lee HC, Cho DY, Lee WY, Chuang HC. Pitfalls in treatment of acute cervical spinal cord injury using high-dose methylprednisolone: a retrospect audit of 111 patients. *Surg Neurol.* 2007;68 Suppl 1:S37-41; discussion S41-32.

29. Qian T, Guo X, Levi AD, Vanni S, Shebert RT, Sipski ML. High-dose methylprednisolone may cause myopathy in acute spinal cord injury patients. *Spinal Cord.* Apr 2005;43(4):199-203.

30. Patki P, Hamid R, Shah J, Craggs M. Fertility following spinal cord injury: a systematic review. *Spinal Cord.* Feb 2007;45(2):187.

31. Weaver LC, Marsh DR, Gris D, Brown A, Dekaban GA. Autonomic dysreflexia after spinal cord injury: central mechanisms and strategies for prevention. *Prog Brain Res.* 2006;152:245-263.

32. Mariano AJ. Chronic pain and spinal cord injury. *Clin J Pain.* Jun 1992;8(2):87-92.

33. Sawatzky B, Bishop CM, Miller WC. Classification and measurement of pain in the spinal cord-injured population. *Spinal Cord.* Jan 2008;46(1):2-10.

34. Strauss DJ, Devivo MJ, Paculdo DR, Shavelle RM. Trends in life expectancy after spinal cord injury. *Arch Phys Med Rehabil.* Aug 2006;87(8):1079-1085.

35. Nandoe Tewarie RD, Hurtado A, Levi AD, Grotenhuis JA, Oudega M. Bone marrow stromal cells for repair of the spinal cord: towards clinical application. *Cell Transplant.* 2006;15(7):563-577.

36. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* Apr 2 1999;284(5411):143-147.

37. Petersen BE, Bowen WC, Patrene KD, et al. Bone marrow as a potential source of hepatic oval cells. *Science.* May 14 1999;284(5417):1168-1170.

38. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve.* Dec 1995;18(12):1417-1426.

39. Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann N Y Acad Sci.* Jun 2001;938:221-229; discussion 229-230.

40. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science.* Dec 1 2000;290(5497):1779-1782.

41. Ohta M, Suzuki Y, Noda T, et al. Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. *Exp Neurol.* Jun 2004;187(2):266-278.

42. Lu P, Yang H, Jones LL, Filbin MT, Tuszynski MH. Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. *J Neurosci.* Jul 14 2004;24(28):6402-6409.

43. Hofstetter CP, Schwarz EJ, Hess D, et al. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci U S A.* Feb 19 2002;99(4):2199-2204.

44. Ankeny DP, McTigue DM, Jakeman LB. Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. *Exp Neurol.* Nov 2004;190(1):17-31.

45. Zurita M, Vaquero J. Functional recovery in chronic paraplegia after bone marrow stromal cells transplantation. *Neuroreport.* May 19 2004;15(7):1105-1108.

46. Nandoe Tewarie RD, Hurtado A, Ritfeld GJ, et al. Bone marrow stromal cells elicit tissue sparing after acute but not delayed transplantation into the contused adult rat thoracic spinal cord. *J Neurotrauma.* Dec 2009;26(12):2313-2322.