Multimodality Treatment of Spinal Cord Injury: Endogenous Stem Cells and Other Magic Bullets

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Acute traumatic SCI initiates a complex cascade of inflammation and ischemia that leads to scar formation. After injury this scar formation provides a strong inhibition to regeneration. Because the overall injury occurs on multiple levels, both spatially and temporally, a multimodality approach to treatment is needed. Only by combining neuroprotective and neuroregenerative treatments can significant advances be made to overcome SCI. Furthermore, new techniques of manipulating endogenous stem cells show great promise in promoting neuroregeneration.

Key Words: neuroprotection, neuroregeneration, spinal cord injury, trauma

Abbreviations Used: 4-AP, 4-aminopyridine; bFGF, fibroblast growth factor beta; EGF, epidermal growth factor; FGF2, fibroblast growth factor 2; NMDA, N-methyl-D-aspartate; SCI, spinal cord injury; Shh, sonic hedgehog

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Each year in the United States, SCI affects 10,000 to 14,000 persons. The mean age at injury is 30 years. Consequently, at any given time, 150,000 to 300,000 people are living with significant disabilities related to SCI. Estimates of the lifetime costs to care for an individual with a SCI range from $325,000 to $1.35 million, and the annual cost to society reaches $8 billion. As long-term care technologies improve, these costs are expected to continue to increase. There have been significant advances in accessibility for persons with disabilities. Nonetheless, the goal of medical science is to overcome the physiological barriers imposed by the injury itself to allow these individuals to regain their preinjury level of neurological function.

The severity of these injuries ranges from complete paralysis to mild myelopathy, depending on the mechanism. Injuries from acute trauma such as automobile accidents tend to garner the most attention, but insidious injuries from degenerative spinal disease are far more prevalent. When treatment of these various types of injuries is considered, it is important to consider the mechanism of injury.

In severe traumatic injuries associated with significant physical force at the time of injury, the initial trauma causes most of the destruction, which is related to shearing and to laceration and disruption of neurons, axons, and supporting tissue (e.g., vascular, connective). After the initial injury, significant scar tissue forms and acts as a barrier to the repair of injured tissue. For such injuries, the ideal treatment should include realignment of the spinal column to minimize further physical trauma to the spinal cord, prevention of sub-

sequent ischemia from the secondary injury cascade, and promotion of neural regeneration.

The same principles apply to lower-impact SCIs (e.g., from degeneration, spinal tumors), but there are significant and important differences in treatment. The first step in treating this type of SCI is to decompress the offending pathology. Because the long-standing compression has led to chronic ischemia, the next step is to prevent further ischemia by promoting adequate tissue perfusion of the spinal cord. Finally, promoting either regeneration or remyelination of the damaged neural elements is needed for further recovery of function.

Until 25 years ago, the prevailing wisdom was that SCIs were irreversible. Consequently, the focus was on helping patients with disabilities to become integrated into society. However, in 1980 one of the first demonstrations of the regenerative ability of injured spinal cord tissue was published.[36] Thereafter, such research expanded exponentially. Although various treatment schemes have been successful in rodent models of SCI, no treatment has yet been effective in humans.

A potential reason for this lack of success has been the focus on finding the 'magic bullet' treatment that will allow an injured spinal cord to recover. The mechanism of SCI is as complex as it is varied, especially the temporal sequence of events after injury. Most likely, a multimodality approach to SCI is needed to make meaningful gains in the clinical treatment of humans. Most research on treatment of SCI falls into two broad categories, which serve as natural starting points for attempting multimodality treatment regimens. The first treatment strategy is to attenuate the secondary injury cascade (neuroprotection); the second strategy is to promote remyelination and regeneration of axons (neuroregeneration).

The secondary injury cascade, which begins soon after the primary injury has occurred, can be influenced by many factors such as hypoxia, hypotension, and the extent of the primary injury (Fig. 1). The initial insult disrupts the microvasculature, which leads to tissue hypoperfusion.[11] The hypoperfusion can be severely ac-

centuated by systemic variables such as pulmonary and cardiovascular dysfunction related to the inability of the spinal cord tissue to autoregulate perfusion after traumatic injury.[40] The resulting profound tissue ischemia persists hours to days after injury. In addition to the initial injury, the ischemia initiates a cascade of cellular destruction due to the breakdown of cellular membranes and to the release of multiple factors such as calcium and glutamate.[1] These factors further potentiate the breakdown of cellular membranes by activating proteases and phospholipases in a positive-feedback loop.

The role ischemia plays in the secondary injury cascade is well studied in animal models.[11] To date, the most effective way to limit the extent of spinal cord ischemia after injury is to limit systemic hypoxia and hypotension. In various experimental animal models of SCI, neuroprotective agents that limit excitotoxicity and membrane breakdown caused by ischemia have been studied extensive-

ly. Significant neurological improvement has followed treatment with sodium-channel modulators, glutamate-receptor blockers, glucocorticoids, and gangliosides.[5,13,20,27] Only a handful of treatments, however, has been tested in human trials of acute SCI. Moreover, the primary issue with spinal cord ischemia is disruption of the vasculature itself. This disruption creates a physical barrier to tissue perfusion. In turn, the barrier limits the ability to deliver pharmacological agents to the site of injury. This limitation is one possible reason why many of these agents are unsuccessful in treating SCI.

Neuroprotection

A controversial treatment for SCI is the use of high-dose methylprednisolone. Glucocorticoids such as methylprednisolone stabilize cellular membranes, reduce vasogenic edema, enhance spinal cord blood flow, alter the concentration of electrolytes at the site of injury, inhibit en-
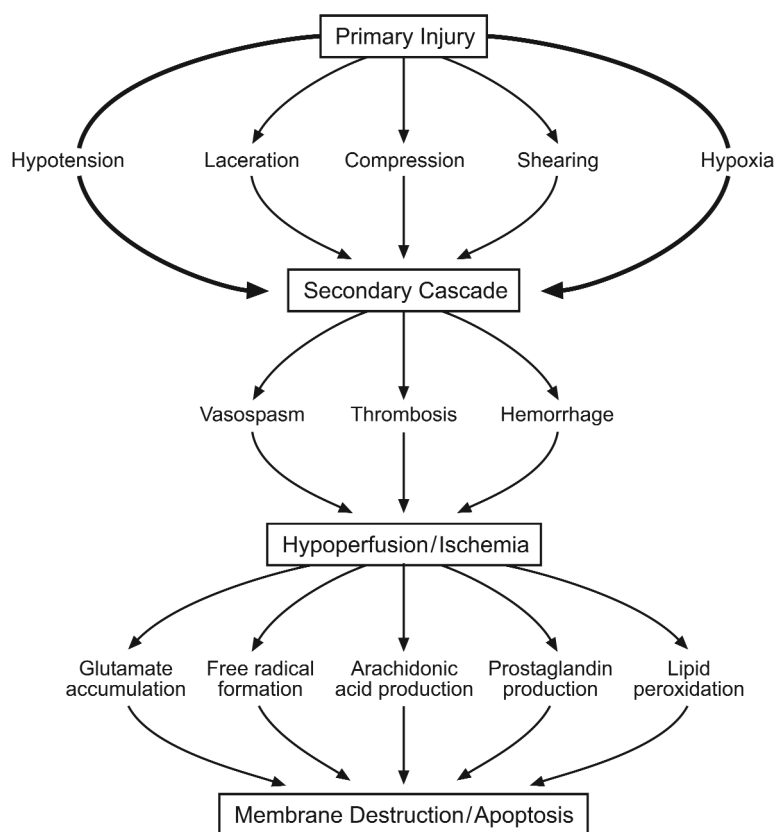

Figure 1. Outline of the primary and secondary injury cascades after acute traumatic SCI.

dorphin release, scavenge damaging free radicals, and limit the inflammatory response after injury.[21] Based on these basic properties of methylprednisolone and on the promising results from animal trials, the first randomized trial in humans was reported in 1984.[6] One year after injury, however, this study showed no differences in the neurologic outcomes of patients receiving low or high doses of methylprednisolone. Subsequent animal studies, however, indicated that the dose used in the trial was too low to produce significant differences in long-term functional outcomes.[9]

To address the issue of underdosing with methylprednisolone in the first trial, a second trial was undertaken with a high dose of methylprednisolone to assess neurological improvement after acute SCI.[7] This trial demonstrated a small but significant improvement in motor scores 1 year after injury compared to a placebo group. However, several aspects of the study have been criticized strongly. The primary complaints were the lack of a standardized assessment of functional outcome (as opposed to basic motor scores) and the use of post hoc analysis to determine statistical significance.[23] A third trial then found that methylprednisolone had a greater benefit if administered within 3 hours rather than within 8 hours of injury.[8] Because of the significant problems associated with these studies, methylprednisolone for the treatment of acute SCI is only considered an option.[18,19] Methylprednisolone has also been associated with medical complications, primarily an increased incidence of infections, gastrointestinal problems, and pulmonary issues.[28] Evidence concerning its long-term effects is mixed.[16]

Other agents tested in human clinical trials include tirilazad, naloxone, and GM-1 ganglioside. The opiate antagonist naloxone was included in the second methylprednisolone trial, but its use was associated with no significant clinical benefit.[7] In the third trial, the 21-aminosteroid tirilazad was compared to methylprednisolone. No benefit was found, but the trial lacked a true placebo group.[8] Two randomized clinical trials have analyzed the effectiveness of the ganglioside

GM-1 on neurological improvement after SCI. The first, smaller study showed a marked improvement in functional neurological outcomes in the GM-1 group compared to the control patients.[15] The larger study failed to detect this improvement. Consequently, ganglioside GM-1 is only considered an option for acute SCI.[14]

Other promising neuroprotective agents are thyrotropin-releasing hormone, the NMDA-receptor antagonist gacyclidine, and the calcium–channel antagonist nimodipine.[26,33,34] These agents were tested in clinical trials to determine their effect on outcomes when used to treat SCI in humans. Unfortunately, none showed any benefit compared to the placebo and all have been abandoned. One agent that has shown promise in human trials is the potassium–channel antagonist, 4–AP. Although 4–AP failed to benefit patients with chronic SCI, this agent may have the potential to stabilize damaged axonal membranes during the acute period of injury.[17]

Several treatments are being developed to provide neuroprotection after SCI. Although these agents have only been tested in animal models of SCI, they represent the next wave for clinical trials in humans. The sodium–channel antagonist riluzole has significantly improved the outcome of SCI in rats. The Food and Drug Administration recently approved its use as a treatment for amyotrophic lateral sclerosis.[37] Attenuation of the inflammatory response after acute SCI has also shown great promise in animal models. When used to treat animals with SCIs, COX-2 inhibitors, ibuprofen, tetracycline, and erythropoietin have all improved functional recovery.[10,22,35,38]

Neuroregeneration

Once the secondary injury has evolved, the process of neuroregeneration begins. Unfortunately, the central nervous system is not a permissive site for neuroregeneration because inhibitors of axonal growth are derived from the formation of scar tissue. The goal for regeneration is to attenuate or overcome

this inhibition to allow repair and regeneration at the site of injury. Several strategies have been used to help the spinal cord to allow regeneration.

One such strategy is to inject activated macrophages into the site of injury to reduce the concentration of inhibitory factors after injury. The macrophages, activated with autologous peripheral nerve tissue, help clean up the cellular debris and damaged myelin that contribute to the strong inhibition to regeneration after injury. Using this therapy in patients with a complete SCI in a Phase I safety trial, three of eight patients improved without significant side effects.[25] This treatment is now being evaluated in Phase II clinical trials at multiple sites worldwide.

Another regenerative treatment being used in clinical trials modifies the cellular cascade that leads to the inhibition of regenerating axons. The activity of the second-messenger pathway that uses the Rho protein in injured axons increases after injury and is partially responsible for the inability of these axons to grow through the glial scar. A Rho antagonist (C3 transferase) that has been developed has a robust ability to allow axonal regeneration and functional recovery in animal models of SCI.[39] This agent (Cethrin, BioAxone, Therapeutic, Inc.; Montreal, Quebec, Canada) is undergoing Phase I/IIa safety and efficacy trials in patients with complete SCIs. As long as 2 weeks after injury, the drug is applied at surgery. It then diffuses across the dura to deliver a high concentration locally at the site of injury.

An exciting treatment potential for promoting neuroregeneration after SCI is stem cell transplantation or stimulation. Although transplantation of stem cells into the injured spinal cord has shown great promise in animal models, work in humans has been limited.[12] One reason for this slow progress is the ethical dilemma inherent when working with embryonic stem cells. Consequently, other solutions, including stem cells derived from bone marrow and stimulation of endogenous stem cells, have been investigated.

Activation and promotion of endogenous stem cells are particularly attractive

because 500,000 and 2 million new cells are produced at the site of injury during the first month after injury.[31] After a contusion injury in animal models, endogenous neural progenitor cells are up–regulated.[24,29,30] Most of these cells originate near the ependyma of the central canal. The greatest level of induction occurs 3 to 7 days after injury. However, most of these cells develop into non–neuronal cells and actually contribute to the inhibition of neuroregeneration. Therefore, this line of research focuses on how to promote endogenous stem cells to develop into cell types that help injured axons to survive and regain function.

The process of differentiation of endogenous stem cells in the adult spinal cord after injury is yet to be determined. Several agents, however, have been used to control the differentiation of these stem cells. Based on existing knowledge, the goal of this treatment is to steer the endogenous stem cells away from the astrocytic pathway and toward a neuronal or oligodendrocytic pathway. By studying the genetic profile of early spinal cord development, proteins such as sonic hedge-hog (Shh) and fibroblast growth factor beta (bFGF) have shown promise in controlling this differentiation. The Shh protein, which is involved in early neuronal differentiation, dramatically increases the number of neuronal progenitor cells in the spinal cord after a demyelination injury.[3,4] In rats with a contusion injury to the spinal cord, the neuronal progenitor cells increase after Shh is administered.[3] The combination of Shh with oligodendrocyte precursors also reduces the amount of cellular damage and improves functional recovery in rodents after SCI
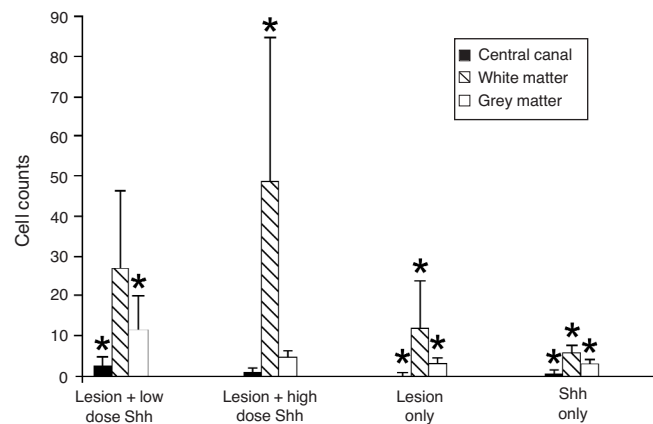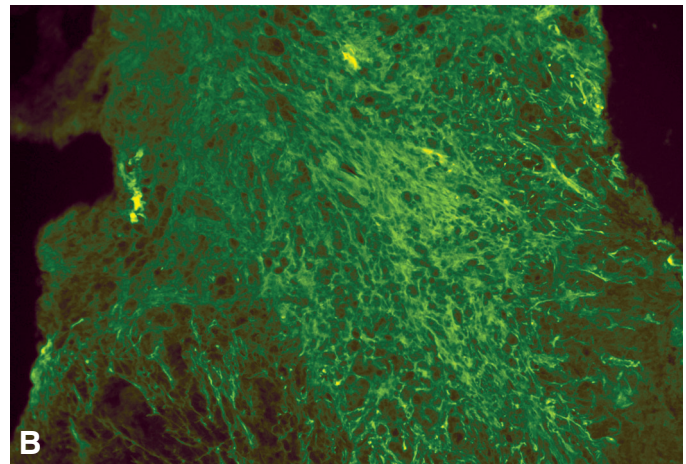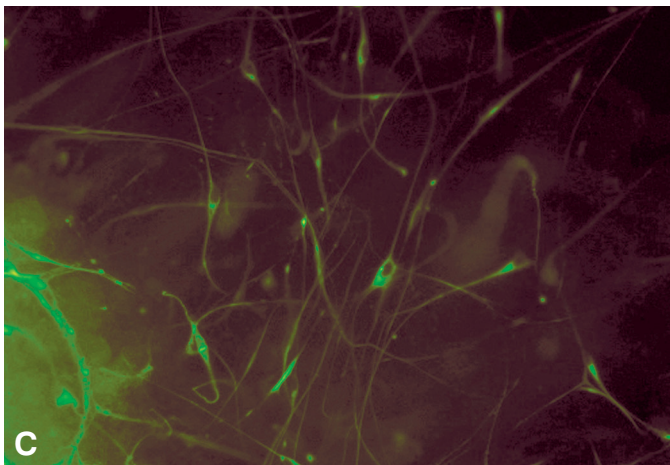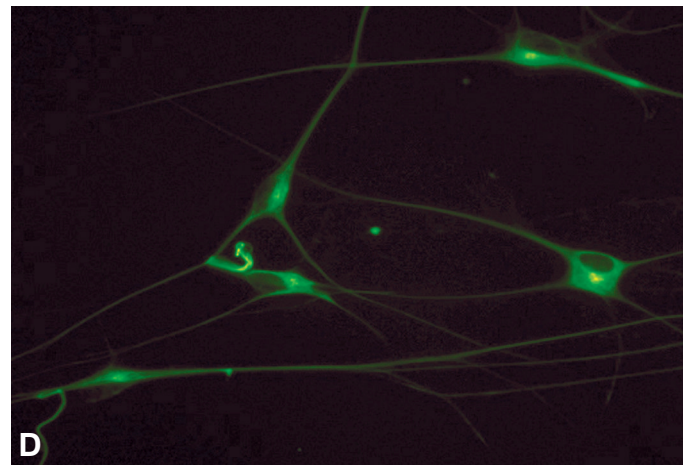

Figure 2. (*A*) Bar graph demonstrates elevation in the cell counts of actively dividing cells in the spinal cord sections of adult rats. Rats with a spinal cord lesion were treated with a low dose (3.0 μL) or high dose (6.0μL) of Shh. Rats without a lesion were given a high dose of Shh. The number of proliferating cells significantly increased after rats with a lesion were exposed to Shh. Data were analyzed with repeated-measures analysis of variance, followed by the Student-Newman-Keuls post hoc t-test (*$p < 0.01$). (*B*) Photomicrograph shows diffuse positivity for nestin in dorsal regions of hyperproliferation. Nestin is an intermediate filament protein found in central nervous system precursors (original magnification, 100x; nestin). (*C*) Low-power (original magnification, 50x, nestin) and (*D*) high-power (original magnification, 200x, nestin) photomicrographs of nestin-positive neural precursors from dorsal explant cultures from the spinal cords of rats that received a contusive spinal cord lesion and were treated with Shh. These primitive-appearing cells characteristically demonstrate bipolar morphologies and are highly motile and proliferative. [From Bambakidis NC et al: Endogenous stem cell proliferation after central nervous system injury: alternative therapeutic options. Neurosurg Focus 19 (3):E1, 2005]. *Used with permission from Neurosurgical Focus.*

(Fig. 2).[2] Likewise in rodents, the expression of bFGF in spinal cord cells increases after traumatic injury. In cell cultures, the bFGF derived from these cells caused them to differentiate into neuronal phenotypes.[41] After a contusion is induced in genetically engineered mice, other growth factors such as EGF, FGF2, neurogenin2 and Mash1 promote neuronal differentiation of endogenous stem cells at the site of injury.[32]

Conclusions

The complexity of the cellular destruction after SCI belies a multimodality approach to treatment. Temporally, the three hallmarks of this treatment are segregated into (1) the acute period during which the best clinical treatment is needed, (2) the subacute period during which neuroprotective treatment is needed, and the (3) delayed period during which neuroregenerative treatment is needed (Fig. 3). The burgeoning field of neuroregeneration, especially the manipulation of endogenous stem cells, may promote significant advances in the treatment of this devastating clinical condition.
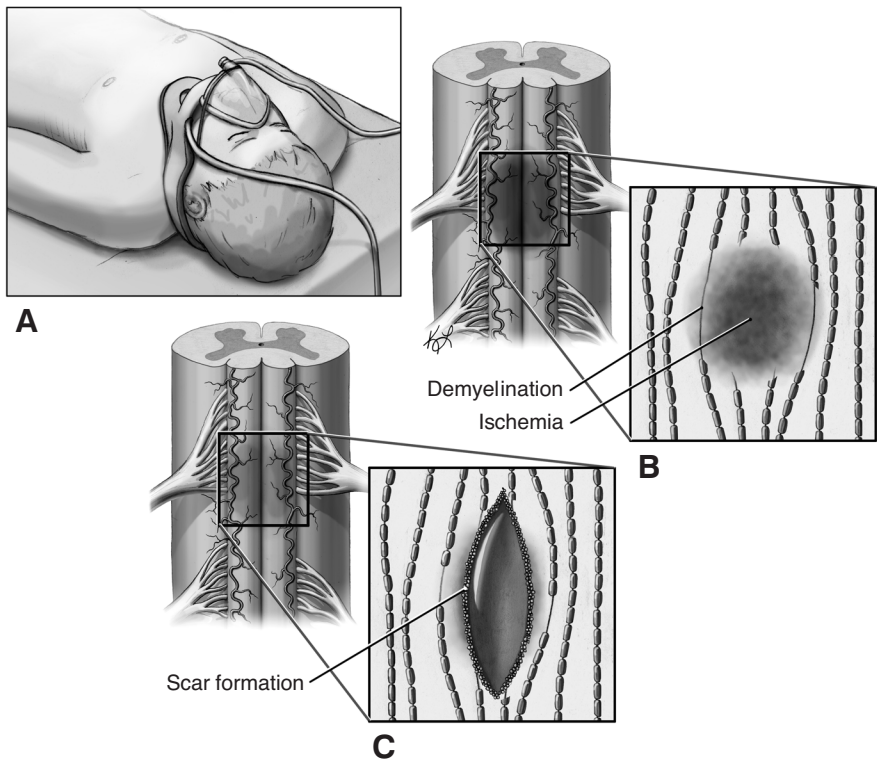

Figure 3. Triphasic treatment paradigm for SCI. (*A*) Illustration shows optimum clinical management during early minutes after spinal cord injury. Spinal protection during prehospital treatment, surgical stabilization, and spinal cord perfusion and oxygenation are key. (*B*) The second step is neuroprotection by the use of methylprednisolone, minocycline, erythropoeitin, riluzole, and 4-AP. (*C*) During the weeks after injury, the last phase includes neuroregeneration using endogenous stem cell promotion, Cethrin, and ProCord (Proneuron Biotechnologies, Inc.; Los Angeles, California) to prevent scar formation.

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