Archives of Medical Research

Archives of Medical Research 55 (2024) 102935

REVIEW ARTICLE

Traumatic Human Spinal Cord Injury: Are Single Treatments Enough to Solve the Problem?

Israel Grijalva-Otero,[a,1,*] and Ernesto Doncel-Pérez[b,1]

[a] *Medical Research Unit for Neurological Diseases, Unidad Médica de Alta Especialidad, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico*

[b] *Neural Regeneration Group, Hospital Nacional de Parapléjicos de Toledo, Servicios de Salud de Castilla-La Mancha, Toledo, Spain*

Received for publication July 3, 2023; accepted December 11, 2023 (ARCMED-D-23-00545).

Traumatic spinal cord injury (SCI) results in partial or complete motor deficits, such as paraplegia, tetraplegia, and sphincter control, as well as sensory disturbances and autonomic dysregulation such as arterial hypotension, lack of sweating, and alterations in skin lability. All this has a strong psychological impact on the affected person and his/her family, as well as costs to healthcare institutions with an economic burden in the short, medium, and long terms. Despite at least forty years of experimental animal studies and several clinical trials with different therapeutic strategies, effective therapy is not universally accepted. Most of the published works on acute and chronic injury use a single treatment, such as medication, trophic factor, transplant of a cell type, and so on, to block some secondary injury mechanisms or promote some mechanisms of structural/functional restoration. However, despite significant results in experimental models, the outcome is a moderate improvement in muscle strength, sensation, or eventually in sphincter control, which has been considered non-significant in human clinical trials. Here we present a brief compilation of successful individual treatments that have been applied to secondary mechanisms of action. These studies show limited neuroprotective or neurorestorative approaches in animal models and clinical trials. Thus, the few benefits achieved so far represent a rationale to further explore other strategies that seek better structural and functional restoration of the injured spinal cord.

Key Words: SCI treatment, SCI structural restoration, Neuroprotection, Neural regeneration, SCI repair.

Introduction

The world's incidence of spinal cord injury (SCI) oscillates between 10.4 and 83 cases per million people annually; one-third are reported to be tetraplegic, and 50% of patients have a complete lesion. The mean age is reported to be 33 years old, with a male preponderance (3.8/1) (1). The Global Burden of Disease Analysis (1990–2019) for SCI reported 0.91 million new cases, 20.64 million prevalent cases, and 6.20 million chronic cases in 204 countries. In this study, falls were the leading cause of SCI (2).

The incidence of complete injuries has decreased slightly compared to incomplete ones, possibly due to improved safety devices and regulatory efforts (3) and advances in prehospital care. Prompt evaluation and treatment in life-preserving institutions, following the principles for rapid and systematic identification of injuries, and treatment initiation according to the Advanced Trauma Life Support (ATLS) guidelines (4) is the current procedure. These include immediate on-site management, monitoring of cardiorespiratory compromise, limitation of spinal movement, transfer to a center capable of providing definitive care and intensive care monitoring, immediate

[1] These authors contributed equally to this work

Address reprint requests to: Israel Grijalva-Otero, Avenida Cuauhtémoc 330, Col. Doctores, Alcaldía Cuauhtémoc, 06720, Mexico City, Mexico; E-mail: igrijalvao@yahoo.com or israelgrijalva1@gmail.com

0188-4409/$ - see front matter.
https://doi.org/10.1016/j.arcmed.2023.102935

management, and correct delimitation of the lesion, including accelerated CT and MRI scans. Also, intervention plans such as pharmacological or surgical treatment (early decompression), early prevention of complications such as gastric ulcer and thromboembolic prophylaxis, and early nutrition (5), implemented in the first 24–48 h and days after the injury.

Despite the slightly decreased incidence of complete injuries compared to incomplete ones due to advances in post-traumatic management and intensive rehabilitation which have improved the functionality and prognosis of SCI, we still do not have consistent therapeutic strategies to significantly improve the paralysis of these patients because SCI is a severe injury caused by multiple and complex set of events, processes, and responses affecting the structure and function of the nervous, vascular, and immune systems, that occur simultaneously and subsequently as a cascade from minutes to months after the initial injury (6–10).

Traumatic SCI is a continuous process resulting from initial mechanical injury and subsequent vascular, tissular, and cellular changes, which can be divided into acute (<48 h), subacute (48 h –14 d), intermediate (14 d to three months), and chronic (>3 months) phases (11). To understand the pathophysiology of traumatic SCI, this continuum process can also be divided into primary and secondary injuries. Primary injury caused by mechanical trauma originates from compression, stretching, laceration, or transection of the spinal cord, which leads to hemorrhage, disruption of the blood-spinal cord barrier and consequently axon severing, membrane rupture, and death of neurons, glia, and endothelial cells (5–9). As part of a continuous process, the primary injury leads to the pathophysiological events of the secondary injury phase, characterized by edema, hypoxia and ischemia, excitotoxicity, electrolyte shifts, free radical production, inflammation, and other multiple alterations, which result in a prolonged period of tissue destruction (5–9).

In the present manuscript, we will briefly describe some important mechanisms of injury in the SCI cascade. We relate the post-SCI events that lead to spinal tissue destruction to the short- or long-term formation of a barrier for regeneration and remyelination, which prevents the restructuring of neuronal networks and the recovery of neural functions. The most relevant individual treatments with drugs or cells used in animal models and/or clinical trials showing different degrees of functional improvement of the injured spinal cord are discussed.

Primary and Secondary Mechanisms of Injury

Generalities on Spinal Cord Injury

The first event is the mechanical injury due to the accident (traffic, fall, violence, etc.). The contusion, compression, or immediate and/or sustained stretching causes blockage of blood circulation in the damaged area and/or rupture of its vessels both macroscopically and microscopically, as well as rupture of axons and cell membranes of the other neural and non-neural cells. As an immediate consequence, the blockage of blood circulation generates edema and an increase in local volume. At the same time, macroscopic and microscopic hemorrhages also increase the local volume of the lesion area. The increase in volume increases the circulatory difficulty due to the secondary compression of the lesion zone and surrounding areas. In addition to the above, the bony elements of the spine can aggravate the already indicated compression and even tear the medullary tissue, causing additional bleeding that leads to progressively greater blockage of blood circulation. Depending on the magnitude of the edema, the bleeding, and the presence of bone elements and even fragments (bone or metal from projectiles) that invade the spinal canal, the degree of involvement of the initially centralized area of injury can extend rostral and caudally, increasing the mechanical damage due solely to the progressive increase in the volume of the injured and adjacent area (12). In addition, depending on the magnitude of the injury mechanism, there may be dislocation of the spine with secondary laceration of the spinal cord and even its rupture, immediately disconnecting the brain centers of the distal portion of the injured spinal cord. These last two scenarios warrant early surgical decompression and spinal stabilization (13–15). The aforementioned phenomena can be classified as the mechanisms of primary injury. Notwithstanding the foregoing, secondary injury is well known as the most important phase of the pathophysiological changes of SCI. This is because it is a destructive and uncontrolled cascade with vascular, cellular, and molecular alterations from minutes to months after the initial lesion, depending on the primary lesion to a greater or lesser extent (16).

Briefly, vascular alterations described above are worse during the secondary phase leading to ischemia-hypoxia of the injury site and around. In addition, the rupture of axons and cell membranes of neurons and other neural and non-neural cells release Ca++, K+, and Na+ ions, as well as neurotransmitters, especially glutamate and protein kinases. Simultaneously with the excitotoxicity developing, a decrease in ATP occurs with the subsequent affectation of the ionic pumps of the cell membranes (increase in intracellular Na+ and Ca++ and extracellular K+) with the blockade of neurotransmission and cytotoxic edema, as well as the production of reactive oxygen species (ROS) that mainly induce lipid peroxidation (LP) of cell membranes. Ischemia-hypoxia also causes blood-spinal barrier dysfunction, which allows blood cell invasion by neutrophils, T-cells, monocytes, etc., and initiation of an inflammatory process including cytokines production as interleukin 1-beta (IL-1β), interleukin 1-alpha (IL-1α), interleukin-6 (IL-6) (5–9).

Cellular Events after Spinal Cord Injury and Cell Responses

A cascade of deleterious processes begins immediately after SCI: ischemia, oxidative damage, edema, and glutamate excitotoxicity, which also contribute to substantial secondary damage. Cell permeabilization, proapoptotic signaling, and ischemic injury due to the destruction of microvascular supply cause additional cell dysfunction and death within minutes of SCI. There is also dysregulation of intracellular calcium in both neurons and glia, leading to activation of calpains, which can cause mitochondrial dysfunction and cell death. Microglia, the resident immune cells, respond rapidly to injury, and their early response is protective. However, microglia rapidly switch to proinflammatory cells and release cytokines, which trigger a cascade of events that leads to the infiltration of peripheral immune cells. Activated microglia retract their cytoplasmic processes and become morphologically indistinguishable from peripherally derived macrophages that enter from damaged blood vessels. Within 30 min of injury, microglia and astrocytes begin to express mRNAs for the inflammatory cytokines IL-1β and tumor necrosis factor-alpha (TNF-α) (17).

Innate Immunity as a Primary Response Mechanism after SCI

After the initial damage of an SCI, there is an infiltration of macrophages and neutrophils towards the injury site due to phagocytosis of the cellular debris and myelin. In the acute phase, neutrophils secrete molecules such as proteases, myeloperoxidases, and ROS. The ROS activate microglia and astrocytes at the site, changing their phenotype to reactive cells. In addition, after phagocytosis, macrophages secrete molecules such as ROS, nitric oxide (NO), macrophage inflammatory proteins (MIPα and β), monocyte chemoattractant protein 1 (MCP-1), CXC motif chemokine 10 (CXCL-10), and act as antigen-presenting cells, activating the expression of type II major histocompatibility complex, MHC-II (18).

The secreted ROS activate the resident microglia in the area in their reactive form. Activated microglia secrete interleukins such as interleukin 1 alpha/beta (IL-1α and β); IL-6; TNF-α; interferon-gamma (IFN-γ), as well as ROS and inducible nitric oxide synthase (iNOS), promoting a pro-inflammatory microenvironment. Likewise, astrocytes also secrete interleukins such as IL-12, IFN-γ, IL-1β, and TNF-α, creating a pro-inflammatory environment in the area of injury (19,16).

Secondary Mechanisms of Injury in Subacute and Chronic SCI

In the subacute phase, interleukins such as IL-6, IFN-γ, and IL-12, promote the rearrangement of the cytoskeleton by polymerization in macrophages/microglia towards a pro-inflammatory phenotype M1, which secretes the chemokines CCL2 and CXCL2, the interleukins IL-12, IL-6, IL-1β and IFN-γ, and molecules such as NO and ROS, maintaining an inflammatory environment that corresponds to a secondary mechanism of injury after a week to years from the initial SCI. In addition, there is a migration of T and B cells towards the site of injury. Effector Th1 lymphocytes are activated in the context of MHC-II and CXCL-10 chemokines; when they reach the lesion area, they maintain an inflammatory state and secrete cytokines, that activate signaling pathways such as nuclear factor-kappa B (NFκB), promoting cell death. These molecules expressed by T lymphocytes are recognized by B cells, activating molecules such as B cell activating factor (BAF), B cell maturation antigen (BCMA), and proliferation induction ligand (APRIL), encouraging an increase in IgG and IgM autoantibodies in the environment (13,16).

In the chronic phase, there is a balance in the system that promotes a change from a pro-inflammatory to an anti-inflammatory environment. In this phase, interleukins such as IL-10 and the transforming factor beta (TGF-β), favor polymerization in the M2 macrophage/microglia phenotype and promote the activation of naive lymphocytes in Th2. Macrophages/microglia M2 secrete anti-inflammatory cytokines such as IL-10, TGF-β, IL-13 and IL-4, and growth factors such as brain-derived growth factor (BDNF), nerve-derived growth factor (NGF), epithelial growth factor (EGF), ciliary growth factor (CNTF), and insulin-like growth factor 1 (IGF-1), which promote a neuroprotective environment in the injury site (20).

On the other hand, Th2 helper lymphocytes also promote an increase in the production of growth factors such as BDNF, NGF and neurotrophins 3, 4, and 5 (NT-3; 4 and 5), prevailing an anti-inflammatory microenvironment that supports cell survival (13).

Chronic SCI Characteristics

In the chronic phase, the fibroglial scar continues to mature, there is axonal demyelination, syringomyelia occurs, and there are also processes of axonal regeneration. When the glial reaction occurs, which results after the injury, there is a recruitment of microglia, precursors of oligodendrocytes, meningeal cells, ependymal cells, and astrocytes to the site of the injury, forming a physical barrier that prevents axonal growth, the fibroglial scar (21).

The glial scar functions by isolating the injury zone from the rest of the tissue and producing factors that cause the area to inhibit axonal growth and prevent aberrant connections. The factors present in the glial scar are tenacins, semaphorins, ephrins, and proteoglycans-chondroitin sulfate (22); these factors are produced by the cells found in the scar, mainly reactive astrocytes and oligodendrocytes.

Therapeutic Strategies on SCI

Over the last 40 years several therapeutic strategies have been developed to improve functional recovery in patients with traumatic SCI, attenuate secondary mechanisms of injury, or favor structural repair. At least 25 different mechanisms of injury have been described, from the acute to the chronic stages (8). There are under investigation many therapeutic agents that mainly target secondary mechanisms as a) neuroprotective strategies (anti-oxidative stress, anti-apoptotic, anti-excitotoxicity, etc.), b) structural repair strategies (neuronal regeneration and axonal connectivity), and c) others (neural stimulation, neuromodulation, computer-brain interfaces, etc.). It also includes non-invasive agents, such as oral or parenteral/systemic drug administration, and invasive procedures, such as *in situ* application of drugs, neurotrophins, cell or biomaterial implantation, etc.

The following sections describe the use of single therapeutic agents in preclinical research and clinical trials. It is known that most of them have more than one mechanism of action, but we will classify each one according to its main activity.

Positive Effects of Single Therapeutic Strategies on Acute-Subacute Phases of SCI. Neuroprotective approach

Neuroprotective therapies focus on impeding or preventing further progression of the secondary injury. There are multiple publications about neuroprotection and functional recovery in animal models and some clinical trials using different types of old and new drug strategies (23–25). Neuroprotective therapies have more than one mechanism of action; however, to facilitate the classification, we will include the most relevant references and treatments.

Early Decompression

Over the past 50 years, laboratory studies have supported the theory that decompressive surgery of the spinal cord after SCI attenuates secondary injury mechanisms and improves neurological outcomes (26–28); however, clinical evidence was not enough to test this hypothesis until almost the past 20 years, when a systematic review suggested that decompression within 24 h resulted in improved outcomes compared with both delayed decompression and conservative treatment (29). The current recommendation is decompressive surgery 24 h after SCI, which has also been associated with an improved neurological outcome of at least a two-grade improvement on the American Spinal Injury Association Impairment Scale at six months follow-up (14).

Excitotoxicity and Ionic Imbalance

Within minutes of primary SCI, the combination of direct cellular damage and ischemia/hypoxia triggers a significant increase in extracellular glutamate, which binds to ionotropic (NMDA, AMPA, and Kainate receptors) and metabotropic receptors with consequent calcium influx inside the cells (7,30). Increased Ca2+ concentration in the cytosol, mitochondria, or endoplasmic reticulum has detrimental consequences for the cell (30,31). A mitochondrial calcium overload can cause apoptotic or necrotic cell death. Ca2+ overload induces intrinsic apoptotic pathways in neurons and oligodendrocytes and causes cell death in the first week of SCI in the rat (13,31). Mitochondrial calcium overload also impedes mitochondrial respiration and leads to ATP depletion, which inactivates Na+/K+ ATPase and increases intracellular Na+ (13,31). The intracellular Na+ excess reverses the activity of the Na+/Ca2+ exchanger, allowing more Ca+ influx (32). Cellular depolarization activates voltage-gated Na+ channels that result in the entry of Cl− and water into the cells along with Na+, causing swelling and edema (33).

Riluzole is an anticonvulsant that works through blockage of the sodium channels, preventing an increase in intracellular sodium and calcium concentrations, thereby inhibiting excitotoxicity. Riluzole significantly reduced tissue loss in rostrocaudal regions surrounding the epicenter of SCI, and also significantly increased retrogradely labeled red nuclei neurons (7,34). Unfortunately, despite promising results in animal models, clinical trials of riluzole have not reached consistent positive functional results, or the benefits have been modest (35,36)

Lipid Peroxidation/Oxidative Stress

Mitochondrial Ca2+ overload activates NADPH oxidase and induces the generation of superoxide by the electron transport chain (13,37). Although $O_2^{\bullet -}$ itself is less reactive, its reaction with the nitric oxide (•NO) radical forms the highly reactive oxidizing agent, peroxynitrite (PN, ONOO–), a crucial player in post-traumatic oxidative damage (38,39). PN can generate multiple highly reactive free radicals, including nitrogen dioxide ($\bullet NO_2$), hydroxyl radical (•OH), and carbonate radical ($\bullet CO_3$), each of which can readily induce LP within the phospholipid membranes of the mitochondrion, leading to respiratory dysfunction, calcium buffering impairment, mitochondrial permeability transition, and cell death (39). In association with LP, amino acids undergo significant oxidative damage from PN and the other free radicals and can nitrate the tyrosine residues of amino acids (38). LP and protein oxidation show an almost identical spatial and temporal pattern, increasing rapidly (as early as 1 h) after spinal cord injury and continuing for at least a week after injury (38).

Methylprednisolone (MP) is an effective inhibitor of free-radical reactions and LP when administered in high doses intravenously (40). MP can also inhibit post-traumatic spinal cord ischemia, support aerobic energy

metabolism, decrease intracellular calcium overload, and reduce calpain-mediated neurofilament loss (41). Despite that, phase III clinical trials were not convincing of its efficacy and safety, and currently, the administration of MP for the treatment of acute SCI is not recommended; it remains an option after balancing the benefits and risks for each patient (5).

Melatonin is a potent scavenger of oxygen and nitrogen reactive species (against hydroxyl and peroxyl radicals mediated oxidative damage), decreasing LP, anti-inflammatory, immuno-enhancing, and modulating circadian rhythmicity (41–43), which has demonstrated benefits in the SCI (44). Preclinical studies have served as a basis for the design of clinical trials to study melatonin's possible usefulness in human SCI (43,44).

Other single drugs with antioxidant activity have demonstrated benefits in animal models of SCI. Besides its immunosuppressive and anti-inflammatory activity, cyclosporin-A (CsA) protects spinal cord-damaged tissue from LP by free radicals and produces significant motor improvement in animals treated with CsA (45,46).

Rats subjected to SCI and treated with ascorbic acid (vitamin C) showed significantly better locomotor scores compared to the control group and also reduced necrosis at the injury site (47,48). Vitamin E has been demonstrated to significantly improve hind limb locomotor function, reduce morphological damage in the injured spinal cord, and significantly reduce indicators of oxidative damage (49). Furthermore, in a comparative study, the administration of alpha-tocopherol enhanced the reparative effects on SCI and was also more effective than ascorbic acid (47).

Considering nutritional supplements, docosahexaenoic acid (DHA) has been described to be neuroprotective in rodent models of SCI when applied within 30 min of compression. At 24 h post-injury, lipid peroxidation, protein oxidation, RNA/DNA oxidation, and cyclooxygenase-2 induction were all significantly reduced. At six weeks, macrophage recruitment and axonal injury were reduced, neuronal and oligodendrocyte survival were substantially increased. Motor recovery was improved from the fourth day and sustained up to six weeks (50).

Apoptosis

Apoptosis represents a programmed, energy-dependent mode of cell death including neurons, oligodendrocytes, microglia, and astrocytes that begins as early as four hours after the injury (neurons and astrocytes) or 24 h (oligodendrocytes and microglia) and reaches a peak at eight hours in neurons, 24 h in astrocytes, and 7–8 d in oligodendrocytes and microglia (51–53). In SCI lesions, apoptosis occurs mainly due to injury-induced Ca2+ influx, which activates caspases and calpain, enzymes involved in the breakdown of cellular proteins (8). Excitotoxins, free radicals, and inflammatory mediators are also known factors that contribute to cell death and stimulate necrosis or apoptosis (54).

To date, several drugs have been tested in both animal models and clinical trials for SCI. Some examples are mentioned below.

Minocycline, a clinically used tetracycline, crosses the blood-brain barrier and prevents caspase upregulation and decreases caspase-3 mRNA expression, in addition to its anti-inflammatory activity, limiting the degree of functional loss after trauma (55). In contusion animal models of SCI, minocycline showed functional and spared tissue improvement. Locomotion scores were significantly higher in minocycline-treated rats than those in vehicle or tetracycline-treated, and lesion size was significantly reduced. Among other effects, minocycline treatment significantly reduced microgliosis, specific caspase-3 activity, and substrate cleavage (55,56).

Calcitriol and metformin act as inhibitors of apoptosis after SCI. Calcitriol, a biologically active metabolite of vitamin D, promotes locomotor recovery after SCI by reducing oxidative stress and inhibiting apoptosis, as well as inhibiting autophagy (57). Independently, metformin is a glucagon-like peptide-1 agonist used as a hypoglycemic agent for the treatment of type 2 diabetes through adenosine monophosphate-activated protein kinase (AMKP) activation. Metformin attenuates SCI by inhibiting apoptosis and inflammation, enhancing autophagy via the mTOR/p70S6K signaling pathway, and promoting functional recovery in SCI rats by activating the Wnt/β-catenin signaling pathway (58,59).

Atorvastatin and simvastatin are two inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Atorvastatin decreases caspase-3 activity by 50% and approximately 20% in apoptotic cells at the injury site, preventing early apoptosis within two hours of administration (60), and acute atorvastatin treatment decreases caspase-3 cleavage in neurons, astrocytes, and oligodendrocytes (61). Simvastatin reduces neuronal apoptosis and improves function and recovery via activation of the Wnt/β-catenin signaling pathway (62)

Ascorbic acid (vitamin C) has been described to reduce caspase-3 in SCI-induced rats; however, its effect was improved when combined with taurine (63). There is no information available about the single therapeutic effect of alfa tocopherol (vitamin E) on apoptosis; however, when associated with vitamin C, it produces a decrease in cleaved caspase-3 (64).

Considering granulocyte colony-stimulating factor (G-CSF), it has been described that suppresses or significantly decreases neuronal apoptosis after SCI and increases expression of the anti-apoptotic G-CSF target gene Bcl-X(L). In addition, G-CSF improves functional connectivity and promotes hindlimb function recovery (65,66). There are also several phase I to III clinical trials with chronic complete and incomplete SCI and subacute incomplete SCI in

which G-CSF promotes motor, sensation, and basic daily tasks (including bladder and bowel management) improvement (67–70).

Retinoic acid (RA) has a neuroprotective role in recovery from SCI. RA has been described as related to the prevention of spinal cord barrier disruption by activating autophagic flux and inhibiting endoplasmic reticulum stress-induced cell apoptosis (71).

Immune Response and Neuroinflammation

As previously described, neuroinflammation starts from the very beginning of the injury in the acute phase due to disruption of blood vessels and macro- and microhemorrhages in the lesion site with extravasation of blood cells, including neutrophils and monocytes. Posteriorly, inflammatory mediators like TNFα and IL-1β and other molecules such as ROS, nitric oxide, histamine, and matrix metalloproteinases that increase the blood-spine barrier permeability appear (72,73). Infiltration by inflammatory cells, continued activation of resident microglia, and release of noncellular mediators (TNFα, interferons, and interleukins) play important roles both deleteriously to further secondary injury and beneficially to the removal of cellular debris and enhancement of the environment for regenerative growth (73–75). Decreased levels of TNFα, IL-1β, and IL-6 might be an indicator of a better neurological outcome after traumatic SCI in patients (75). For a more detailed explanation of the complex immune response and neuroinflammation in SCI, see Alizadeh A, et al. (13).

Minocycline is a CNS-penetrating tetracycline antibiotic that inhibits microglial activation and downregulates proinflammatory cyclooxygenase-2, TNF-a, and IL-1β. Both preclinical and clinical studies of acute minocycline treatment have shown improved behavioral outcomes, especially improved motor scores (76).

Methylprednisolone, in addition to inhibiting free-radical reactions and lipoperoxidation, acts on cytoplasmic receptors to upregulate anti-inflammatory factors and interfere with the actions of proinflammatory cytokines, arachidonic acid metabolites, and adhesion proteins when administered at high doses within eight hours of injury. As it was said before, methylprednisolone remains as a therapeutic option (76,77).

Several other therapeutic strategies are associated with a reduction in inflammatory response, inhibition of apoptosis, increased tissue sparing, and improved motor recovery in animal SCI studies. CsA is a potent and selective immunosuppressive agent that, due to its mechanism of action, can be used to inhibit the inflammatory response, and act as a neuroprotector agent after SCI (46).

Immunization with neural-derived peptides (INDP) is another beneficial therapeutic strategy in either acute or chronic SCI, that improves motor and sensory function. After moderate acute injury, INDP elicited a pattern of genes characterized by a significant reduction in IL6, IL1β, and TNFα but with an increase in IL10, IL4 (78,79). After a chronic injury, INDP induced a significant production of the anti-inflammatory proteins IL-4 and IL-10 (80).

Supplements such as trace elements and minerals with anti-inflammatory activity have shown positive results in animal models of SCI. DHA is neuroprotective in rodent models of SCI, significantly reducing lipid, protein, and RNA/DNA oxidation and the induction of cyclooxygenase-2 by i.v. DHA also has significant locomotor recovery (50,79,81). Zinc has a protective effect on SCI by inhibiting oxidative damage and NLRP3 inflammation, promoting motor function recovery after the lesion (79,82).

Treatment with D-carnosine, but not L-carnosine significantly reduced the degree of spinal cord inflammation and tissue damage, neutrophil infiltration, and proinflammatory cytokines, among other activities, and significantly ameliorated the loss of limb function after a compression SCI (83).

It has been described that gut dysbiosis impairs recovery after SCI (84) and that reprogramming the gut microbiota by fecal microbiota transplantation improves locomotor and gastrointestinal function in SCI mice, possibly through the anti-inflammatory functions of short-chain fatty acids (85).

Drugs such as Rolipram, Imatinib, Atorvastatin, Metformin, and Sumatriptan, also inhibit inflammation and apoptosis, and improve locomotor recovery in animal SCI studies (7,86–88).

Positive Effects of Single Therapeutic Strategies in the Intermediate-Chronic Phase of SCI. Restorative Approach

Because the final repair effect after SCI is often unsatisfactory due to the limited ability to regenerate nerves, the restorative approach implies structural repair strategies, including cell replacement, axonal regeneration and connectivity, remyelination, and others that support the functional improvement of the injured spinal cord. As mentioned earlier in the section on neuroprotection, there are also several publications that address repair methods and functional recovery in animal models and some clinical trials using different kinds of cell transplantation, biomaterials, and substances that enhance and direct axonal regeneration, connectivity, and remyelination. Most of these strategies have more than one way to improve structural and functional changes. We will briefly describe some of them as individual treatments.

Cell Transplantation

Transplanted cells replace lost and damaged cells and tissues due to primary and secondary mechanisms of the lesion and because they secrete neurotrophic factors that modulate the immune response and local microenviron-

ment, and provide the substrate and support needed for axon regeneration and remyelination (89).

Schwann cells are an important component of nerve regeneration in the peripheral nervous system (90). In animal models of SCI Schwann cells, among other functions, they promote remyelination, provide growth factors and extracellular matrix components, and enhance axon regeneration in the central nervous system, improving motor and sensory function (91). In a clinical trial, autologous Schwann cell transplantation was shown to improve motor, sensory, and autonomic functions in patients (92), and a Phase I clinical trial in patients with chronic SCI showed safety (93).

Mesenchymal stem cells (MSC) are self-renewing cells with multiple differentiation potentials (94). MSC promotes tissue protection and neural repair by secreting neurotrophic factors and regulating immune responses (regulating macrophage phenotype M1 to M2 and reducing inflammatory cell infiltration), inhibiting apoptosis, promoting angiogenesis, and regenerating axons and myelin (95–97). Although positive results have been reported in preclinical studies for the treatment of SCI, the therapeutic effect in clinical studies oscillates between significant and non-significant functional improvement (98,99).

Olfactory ensheathing cells (OECs) are specialized types of glial cells present in the olfactory mucosa and olfactory bulb. OECs have shown several beneficial effects in *in vitro* and in animal studies such as secreting neurotrophic factors, promoting angiogenesis and phagocytosis, regulating local immune responses, producing extracellular matrix to support and guide axon growth and improving motor function (100,101). Although OECs are safe and effective in a limited number of clinical trials, more such studies, especially randomized clinical trials, are needed to confirm their safety and efficacy (100,102).

Neural stem cells (NSCs) are self-renewing cells with multiple differentiation potentials, ranging from neurons that can act as relay neurons to integrate into damaged neural circuits to oligodendrocytes that can form myelin sheaths and promote regeneration and repair of axons and signal transduction (89,103,104). NSCs reduce neuronal apoptosis, inhibit neuroinflammation, and promote autophagy (105), and NSC cell grafts form extensive synaptic networks that integrate with host circuits after SCI (106). Few clinical trials using NSCs have been reported whether for subacute or chronic, cervical or thoracic SCI (107–110). They all demonstrated safe and well-tolerated transplantations and surgical procedures with no additional damage, and a modest neurological benefit at the end of the follow-up (107–110).

Other types of cells are also being studied in search of more therapeutic alternatives. Induced pluripotent stem cells (iPSCs) are self-renewing and have multi-differentiation potential, which are generated by genetic modification and reprogramming of differentiated somatic cells (111). Oligodendrocyte precursor cells (OPCs) can be rapidly activated after SCI, massively proliferate, and differentiate into oligodendrocytes to promote remyelination (112). Genetically modified cells can more efficiently express the required protective nerve growth factor, enhance the differentiation of neural stem cells or progenitor cells into neurons, and improve the survival rate of transplanted cells, thereby improving the efficacy of cell therapy and promoting regeneration and repair of SCI (89).

Axon Regeneration and Remyelination Supporters

Preservation of neurons is important for functional recovery after a CNS injury, including the spinal cord, through adequate connectivity supported by preserved healthy axons. Injured CNS myelin contains growth inhibitory molecules that impede the regeneration of injured axons (113). One way to solve this problem is to neutralize myelin-associated inhibitors (MAIs) in the injured spinal cord, such as Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte-myelin glycoprotein (OMgp), which are known to prevent axonal regeneration. Antagonism of MAIs through vaccination with protein or DNA vaccines targeting Nogo, MAG, OMgp, and their co-receptors enhances sprouting and regeneration of lesioned axons in animal models of SCI (114–117). Another approach is to target downstream intracellular signaling pathways within the growth cone, such as the Rho/Rac pathway. Rho antagonists such as C3-exoenzyme, fasudil, Y-27532, and ibuprofen improved locomotor outcomes in animal models of SCI (7), and some of these approaches have been translated into human studies. C3-like enzymes with improved permeability characteristics (BA-210 + Tisseel, Cethrin) and ibuprofen have been tested in human clinical trials (118). Although an open-label Phase I/IIa clinical trial with BA-210 or VX-210 (trademarked as Cethrin®) showed positive clinical changes mainly in complete cervical injuries, a randomized, double-blind, placebo-controlled Phase 2b/3 trial to evaluate efficacy and safety of the Rho inhibitor VX-210 in patients after acute traumatic cervical SCI was ended early due to failure to meet the primary efficacy end-point, with no statistically significant difference in change from baseline in the upper-extremity motor score at six months after treatment between the VX-210 and placebo groups (119,120).

Remyelination is a regenerative process that occurs spontaneously but is often insufficient to restore lost axonal function and improve neurological deficits after demyelination. Remyelination in the CNS is mainly mediated by adult oligodendrocyte progenitor cells (OPCs) (121). One way to improve remyelination is through several potential small molecules such as benztropine, a muscarinic antagonist, that induces OPC differentiation (122). Clemastine, another antimuscarinic compound also affected oligoden-

drocyte differentiation and myelination. An opioid receptor (KOR) agonist, *U*-50488, is an enhancer of the differentiation and myelination of oligodendrocytes in cell culture; miconazole and clobetasol have also been identified as inducers of oligodendrocyte maturation (121). Unfortunately, these substances have not been tested in SCI. The second way to improve remyelination is cell transplantation in animal models of SCI such as OPCs. In addition to being a substrate for remyelination, OPCs regulate local immune function, secrete a variety of nutritional factors, cytokines, and chemokines, among others, and also have functional recovery effects (89,123). OPCs have also been considered in human transplantation. There is an open-label, dose-escalation, multicenter clinical trial with C4-7 subacute AIS grade A or B in patients with SCI, who received a single dose of OPC administered by intraparenchymal injection into the spinal cord at the site of injury. At one-year follow-up, 21/22 (96%) of the intention-to-treat group recovered one or more levels of neurological function on at least one side of their body, and 7/22 (32%) recovered two or more levels of neurological function on at least one side of their body (124).

Neurorehabilitation is another tool in the treatment of several neurological disorders, not because of the concept of focusing on the restoration of neurological function by making the best use of the residual function to replace what has been lost as much as possible (125), but because neurorehabilitation enhances neural plasticity, especially when started as early as possible (126,127). Exercise as part of neurorehabilitation procedures has shown different ways to improve structural and functional changes in SCI animal models: improving modulation of the mTOR pathway (responsible for differentiation, survival, proliferation, and protein synthesis), blocking or decreasing its negative regulator, phosphatase, and tensin homolog (PTEN), through increasing levels of microRNAs (miR21 and miR199a-3p), which was correlated with a significant increase in mTOR mRNA and an increase in the level of phosphorylated S6 expression in spinal interneurons (126). The mTOR pathway plays a critical role in regulating the regenerative capacity of neurons (128). Exercise after SCI also increases the mRNA of BDNF, GDNF, and neurotrophin 4 (NT-4) (126). BDNF is a powerful neurotrophic factor known to induce plastic changes that regulate neuronal growth, excitability, and even regeneration (129). Bose PK, et al, using hind limb exercise (cycling and treadmill training) after a spinal cord contusion, reported no spasticity in either group and an increased rate of recovery of limb placement (130). In addition, exercise has been described to modulate gene expression in injured neurons, resulting in increased regeneration (127). Therefore, exercise as an essential component of neurorehabilitation, especially early rehabilitation, is a neuroprotective and regenerative agent. Because it is a non-invasive strategy, it should be considered an indispensable strategy for the treatment of several CNS diseases, including SCI (131–133).

Biomaterials

After SCI, during the intermediate and chronic phases, the scar formed by astrocytes matures, cyst formation, Waller's degeneration, nerve demyelination, inflammatory responses, and local inhibitory microenvironmental substances are altogether the main obstacles to axon regeneration and neural circuit repair. In addition to cell transplantation, axonal regeneration, and remyelination supporters, biomaterials are an additional strategy aid for restorative therapeutic procedures to improve structural and functional recovery after SCI. Biomaterials are used to mechanically stabilize the injury site and provide an environment for host cell interactions, physically fill SCI-associated cavities, reconstitute the extracellular matrix (ECM), and bridge the injury to guide axonal growth across the area (134–137).

Biomaterials used to treat SCI can be in the form of conduits, sheets, scaffolds, fibers, particles, or hydrogels (137). Biomaterials can be used to deliver therapeutic agents and cells (134) and to guide axonal regeneration as channels, fibers, scaffolds, and magnetic microgels (137). The use of hydrogel to deliver anti-inflammatory drugs is appropriate for the acute phase of SCI and the use of fibers and conduits to achieve guided neural regeneration at later stages.

However, not all biomaterials are adequate for use in animal models and potentially in humans. Essential elements of a good biomaterial (functional biomaterial) include good biocompatibility, adequate biodegradability, and low immunogenicity (138). According to their main components and preparation methods, functional biomaterials are natural (agarose, collagen, gelatin, chitosan, alginate, fibrin, hyaluronic acid, and extracellular matrix), synthetic (degradable polymers such as PGA, PCL, PLA, PLGA, and PEG; non-degradable polymers such as PHEMA and PHPMA; synthetic polypeptide molecules, and conductive polymers), mix/composite/hybrid (synthetic and natural biomaterials combining desirable properties of both types of materials), and micro/nanomaterials (89,137,139,140).

Because biomaterials have mainly been used as complementary or adjuvant treatments for SCI and other neurological traumatic, ischemic, or degenerative diseases, we will briefly mention some examples as a single treatment for SCI.

Polyethylene glycol (PEG) is a synthetic biocompatible polymer with many useful properties for the development of therapeutics for the treatment of SCI. PEG reduces the inflammatory response, inhibits vacuole and scar formation, mitigates oxidative stress, promotes and guides axonal regeneration, and restores synaptic connections with target tissues, thereby promoting spinal cord repair and im-

proving motor function (141,142). In the search for strategies to bridge large tissue gaps after acute or chronic SCI, PEG was tested. A chronic spinal cord lesion was created by a complete spinal cord transection five weeks before treatment. Then, after the microsurgical scar resection, the resulting cavity was filled with polyethylene glycol (PEG 600), which was found to be an excellent substrate for cellular invasion, revascularization, axonal regeneration, and even compact remyelination *in vivo* (143). In another document that investigated the feasibility of a system of modular hydrogel tubes to promote bidirectional regeneration after SCI, porous hydrogel tubes fabricated from PEG were implanted into a T9-10 lateral hemisection mouse model. The implanted tubes had good apposition and integration with the host tissue; the glial scar was significantly reduced compared to control, which allowed axon growth along the inner and outer surface of the tubes, and approximately 30% of the axons within the tube were myelinated, which improved functional recovery compared to control (144).

Another interesting biomaterial tested is the poly (2-hydroxyethyl methacrylate) (pHEMA). The pHEMA scaffold implanted in the dorsal column, using fluorescent immunohistochemistry to quantify glial scarring, showed reduced intensity compared to lesion controls for GFAP and the chondroitin sulfate proteoglycan neurocan, one of the major inhibitory molecules for axon regeneration in nerve injury (145).

Despite the high number of publications on biomaterials in animal models of disease, their application in humans is limited and should follow international rules (ISO 10993-1:2018(E) (146).

Other Single Strategies Approach

There are other single therapeutic strategies with positive structural and functional results that have not been included above. We will briefly mention some successful results.

In addition to minocycline, there are several other antibiotics from different families with therapeutic effects on SCI, such as macrolides, β-lactams, and dapsone, which reduce inflammatory microglial activity, promote autophagy, inhibit neuronal apoptosis, and modulate SCI-related mitochondrial dysfunction, improving SCI sequels and complications (147,148).

Immunotherapy has now expanded into vast areas of medicine, including the treatment of infectious diseases, allergies, inflammatory diseases, autoimmune conditions, cancer, and CNS diseases such as SCI (149–151). As we mentioned before, research in SCI has continued with good and bad results considering axonal regeneration with MAI antagonists through vaccination with protein or DNA vaccines (150–153). Elezanumab is a promising treatment consisting of immediate or delayed administration of a high-affinity repulsive human guide molecule A (RGMa)-specific monoclonal antibody. Elezanumab promoted neuroprotection and neural tissue axonal repair, regeneration and plasticity, including the formation of neural tissue, caudal synaptic connections to the site of injury, and improvements in various functional tests in SCI rats (153).

As mentioned above, immunization with neural-derived peptides, such as A91, has shown protective T cell-mediated autoimmunity, reduced proinflammatory response, and apoptosis, increased neurogenesis, and promoted the production of neurotrophic factors (NT-3 and BDNF) at both early and late phases of traumatic SCI (tSCI). In addition, immunization with A91 improves functional recovery of both acute and chronically injured rats (80,154,155).

Monocyte locomotion inhibitory factor (MLIF) is a pentapeptide produced by Entamoeba histolytica. In spinal cord-injured animals, reducing iNOS gene products and a significant upregulation of IL-10 and TGF- β expression at the site of injury reduces the nitric oxide concentration and LP levels in the systemic circulation. Also, MLIF improved the rate of motor recovery, which correlated with increased survival of ventral horn and rubrospinal neurons, confirming the neuroprotective effects conferred by MLIF after SCI (156).

More recently, robotic devices have been developed for gait rehabilitation in paraplegic incomplete patients with SCI or for assisted upper extremity training in individuals with cervical SCI. General evidence suggests that robot-assisted interventions are safe and feasible and may reduce active assistance by therapists. In patients with chronic incomplete SCI with independent stepping ability at baseline, robotic exoskeleton training can improve clinical ambulatory status (157), and compared to overground walking, it results in lower scores of fatigue, mental effort, and discomfort and improvements in the walking economy due to decreased energy costs with increased speed and workload (158). In the case of robot-assisted upper extremity training for individuals with cervical SCI, hands-on therapist assistance may be reduced (159). Future research is needed to determine the optimal device and to standardize the type of intervention to evaluate the role of robot-assisted training in individuals with SCI (158,160).

In addition, there are several other new single therapeutic strategies with good or excellent results. One unusual drug showed important benefits in an SCI rat model: the stable gastric pentadecapeptide BPC 157. The beneficial effects of the stable gastric pentadecapeptide BPC 157 have been reported in several organ systems, and recently in CNS disorders, including rat models of stroke and hippocampal ischemia/reperfusion injury, catalepsy and schizophrenia, and spinal cord injury (161). In a rat model of spinal cord compression, the application of BPC 157 showed advanced healing and functional recovery (counteracted tail paralysis). Compared to control rats, those treated with BPC 157 intraperitoneally 10 min post-injury, presented only discrete edema and minimal hemorrhage at

the injured site, improved tail motor function and spasticity, and at the microscopic level, a reduction in axon and motoneuron loss and an increase in the number of large myelinated axons (162). In another study that tested BPC 157 at early post-injury course, immediate effect (intraperitoneal injection), delayed post-injury course, immediate effect (intragastric administration), and delayed post-injury course, long-term effect (therapy started and continuously administered orally drinking water from day 4 to one month after injury), the main results include hematoma reduction and spinal cord swelling in both early and delayed (four days) application; an increase in Nos1, Nos2, and Nos3 levels 30 min post-injury (early); and in the delayed post-injury course, long-term effect, BPC 157 rats rapidly presented tail function, and histologic study 30 d after the injury showed no demyelination process (162). Future research is needed to confirm these results and test them in clinical trials.

Despite the numerous studies published on therapeutic strategies in tSCI, only some of them have been considered in randomized clinical trials. A quantitative systematic review published in 2020 reported a few potential substances that may improve neurological outcomes in acute SCI: MP, vitamin D associated with progesterone, and erythropoietin and their potential benefits were modest in the studies evaluated, and further randomized clinical trials with large patient samples are needed for routine clinical use (163).

Perspectives and conclusions

The structural and molecular pathology of SCI poses critical challenges for successful neural repair that remain to be overcome despite the efforts of the scientific and clinical communities. A better understanding of the complex biological processes, particularly in human cases of SCI, will be essential for the development of highly effective therapies (9). The first challenge is to effectively inhibit the spread of secondary injury cascades. The second challenge is to regenerate the injured spinal cord and restore neural connectivity (7).

To date, the lack or limited efficacy of treatments for SCI is due to multiple factors that simultaneously and progressively affect the injured spinal cord and the general condition of the patient. These factors include external factors (primary mechanisms), lesion-specific factors (secondary mechanisms), patient health status, and factors related to the experimental treatment being used. They all contribute to the complexity of treating human SCI.

External injury factors include the type and severity of the lesion, which can range from contusion with or without ligament rupture, contusion or compression with ruptured ligaments and bony structures with or without vascular injury (hemorrhage), to partial or complete laceration and ruptured of soft and bony tissues, vessels, and spinal cord tissue. As noted above, the injury factors themselves involve secondary injury mechanisms that have been discussed in more detail in the first part of this review. Important patient factors include the level of injury (since the more cephalic the lesion occurs, the more motor, sensory, and autonomic functions are affected), comorbidities, age, associated injuries sustained at the same time as the tSCI, and the individual's response to the trauma. Finally, the factors associated with any experimental treatment should consider that the biological processes involved in the recovery of sensory, motor, and sphincter functions and the voluntary retraining of motor tasks are so diverse that single treatment strategies aimed at reducing some of the secondary mechanisms of injury may be insufficient. This may be because human SCI is highly heterogeneous, and no animal model or experimental paradigm replicates all its aspects.

Since experiments with animal models do not match the complexity of tSCI in humans, we face significant challenges related to interspecies variation and preclinical experimental models. For example, while rat SCI is more pathologically similar to the human case than mouse SCI, mice have been more widely used in preclinical studies (137). Therefore, the use of multiple animal models is suggested, considering the general perception that a potential treatment is more robust if its efficacy can be demonstrated in more than one animal species, especially large animal and primate models of tSCI (7). Given the heterogeneity of human SCI, in which the spinal cord can be variably damaged by many different mechanical forces (e.g., distraction, shear, contusion), it has been suggested that a potential treatment is more robust if its efficacy can be demonstrated in experiments that employ different injury models (e.g., contusion, clip compression, etc.) (7).

An important aspect to consider is the experimental treatment, as each therapeutic proposal tested addresses one or some aspects of the secondary mechanisms of injury. Treatment doses may be insufficient to produce the expected benefit, or there may be a therapeutic window that limits efficacy, or because therapeutic maneuvers were applied before or after the time, they would have produced maximum benefit. To solve these types of problems, Kabu S, et al. (7) propose the following: demonstration of a dose-response effect which suggests that the efficacy is indeed attributable to the therapy, and that the efficacy can be achieved with realistic human doses (useful for drug therapies and to some extent for cell therapies); demonstration of clinically meaningful efficacy, not just "statistically significant", but changes that show a noticeable improvement in locomotion is evident; the timing and window of therapeutic efficacy considering that the experimental treatment should be close to the human reality (therefore, treatments should be shown to be effective in experimental studies when administered after some sort of delay from the time of injury); adequate pharmacokinetic/pharmacodynamic properties to deliver a therapeutic

dose or maintain its effect at the trauma site to promote neuroprotection, regeneration and recovery; and overcoming drug resistance due to P-glycoprotein overexpression, which creates pharmacoresistance in treatments targeting neurological diseases.

Many obstacles to the development of successful treatments for SCI remain to be addressed, including reproducibility of the therapeutic paradigm, variations in the location and severity of SCI among patients, and clinical variability and recognition of the appropriate timing for treatment intervention (137). Different types of tSCI, patient conditions, including age and underlying diseases, and injury severity, including different lesion volumes, may be suitable for different treatment modalities (89). Therefore, more clinically representative models should be considered and developed. There are also differences in biological cues between animals and humans; hence, extrapolation of results can be challenging (137).

The heterogeneity of tSCI is reflected at the cellular level, as we mentioned above, in the cellular events and cellular responses that develop after injury. Therefore, novel methods such as single-cell RNA sequencing combined with multi-omics analyses (genomics, transcriptomics, epigenetics, proteomics, and metabolomics) are important to find alternative mechanisms in the cellular pathophysiological response and new targets for the treatment of SCI (164).

The ongoing progress seen in neural tracing procedures, electrophysiological techniques, as well as imaging hardware and software have improved our understanding of neural circuit plasticity after SCI and the importance of propriospinal circuits in restoring neural connectivity. At the same time, however, the increasing knowledge emphasizes our need for greater control over the processes that govern the rewiring of pathways (11).

While acute neuroprotective interventions are crucial to mitigate secondary injury, therapeutic neuroregenerative approaches are required to help the millions of patients with chronic post-injury disability (76). Currently, functional recovery after SCI is a long process that may take years, and it is unlikely that a full recovery will ever be achieved (137). There is a pressing and urgent need for therapies to improve the neurological outcome of patients with SCI. The clinical evaluation of therapies that show promise in the laboratory setting is a significant challenge that requires considerable resources and time. Failure in such clinical trials is very costly and discouraging for the scientific community, patients, and potential investors (7).

Single treatments for SCI repair remain an option after being used for the last forty years. However, they must include detailed experiments that consider all the aspects mentioned before, as well as multiple animal models (including large animals), different types of lesions, demonstrate dose-response effects, etc. Conversely, if there is a belief that individual therapies are unlikely to cure SCI, we should use the same carefully designed experiments described for individual treatments but follow a combined approach involving neuroprotective and neurodegenerative mechanisms, and targeting multiple pathways through a multifactorial therapeutic approach that addresses the complexity of SCI.

Declaration of Competing Interest

Both authors declare no conflicts of interest

Funding

The present work did not receive any funding.

Acknowledgments

We want to thank Dr. Gabriel Guízar-Sahagún for his revision of the manuscript and his valuable comments. This review is by the Framework Agreement for Research Collaboration, signed between the Castilla-La Mancha Health Service (SESCAM) and the Instituto Mexicano del Seguro Social (IMSS).

References

1. Wyndaele M, Wyndaele JJ. Incidence, prevalence, and epidemiology of spinal cord injury: what learns a worldwide literature survey? Spinal Cord 2006;44:523–529. doi:10.1038/sj.sc.3101893.
2. Guan B, Anderson DB, Chen L, et al. Global, regional and national burden of traumatic brain injury and spinal cord injury, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. BMJ Open 2023;13:e075049. doi:10.1136/bmjopen-2023-075049.
3. Devivo MJ. Epidemiology of traumatic spinal cord injury: trends and future implications. Spinal Cord 2012;50:365–372. doi:10.1038/sc.2011.178.
4. Galvagno SM Jr, Nahmias JT, Young DA. Advanced Trauma Life Support® Update 2019: Management and Applications for Adults and Special Populations. Anesthesiol Clin 2019;37:13–32. doi:10.1016/j.anclin.2018.09.009.
5. Witiw CD, Fehlings MG. Acute Spinal Cord Injury. J Spinal Disord Tech 2015;28:202–210. doi:10.1097/BSD.0000000000000287.
6. Anjum A, Yazid MD, Fauzi Daud M, et al. Spinal Cord Injury: Pathophysiology, Multimolecular Interactions, and Underlying Recovery Mechanisms. Int J Mol Sci 2020;21:7533. doi:10.3390/ijms21207533.
7. Kabu S, Gao Y, Kwon BK, et al. Drug delivery, cell-based therapies, and tissue engineering approaches for spinal cord injury. J Control Release 2015;219:141–154. doi:10.1016/j.jconrel.2015.08.060.
8. Oyinbo CA. Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade. Acta Neurobiol Exp (Wars) 2011;71:281–299.
9. Rowland JW, Hawryluk GW, Kwon B, et al. Current status of acute spinal cord injury pathophysiology and emerging therapies: promise on the horizon. Neurosurg Focus 2008;25:E2. doi:10.3171/FOC.2008.25.11.E2.
10. Venkatesh K, Ghosh SK, Mullick M, et al. Spinal cord injury: pathophysiology, treatment strategies, associated challenges, and future implications. Cell Tissue Res 2019;377:125–151. doi:10.1007/s00441-019-03039-1.

11. Katoh H, Yokota K, Fehlings MG. Regeneration of Spinal Cord Connectivity Through Stem Cell Transplantation and Biomaterial Scaffolds. Front Cell Neurosci 2019;13:248. doi:10.3389/fncel.2019.00248.
12. Leonard AV, Thornton E, Vink R. The relative contribution of edema and hemorrhage to raised intrathecal pressure after traumatic spinal cord injury. J Neurotrauma 2015;32:397–402. doi:10.1089/neu.2014.3543.
13. Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Traumatic Spinal Cord Injury: An Overview of Pathophysiology, Models and Acute Injury Mechanisms. Front Neurol 2019;10:282. doi:10.3389/fneur.2019.00282.
14. Fehlings MG, Vaccaro A, Wilson JR, et al. Early versus delayed decompression for traumatic cervical spinal cord injury: results of the Surgical Timing in Acute Spinal Cord Injury Study (STASCIS). PLoS One 2012;7:e32037. doi:10.1371/journal.pone.0032037.
15. Wilson JR, Singh A, Craven C, et al. Early versus late surgery for traumatic spinal cord injury: the results of a prospective Canadian cohort study. Spinal Cord 2012;50:840–843. doi:10.1038/sc.2012.59.
16. Anwar MA, Al Shehabi TS, Eid AH. Inflammogenesis of Secondary Spinal Cord Injury. Front Cell Neurosci 2016;10:98. doi:10.3389/fncel.2016.00098.
17. Hellenbrand DJ, Quinn CM, Piper ZJ, et al. Inflammation after spinal cord injury: a review of the critical timeline of signaling cues and cellular infiltration. J Neuroinflammation 2021;18:284. doi:10.1186/s12974-021-02337-2.
18. Buzoianu-Anguiano V, Torres-Llacsa M, Doncel-Pérez E. Role of Aldynoglia Cells in Neuroinflammatory and Neuroimmune Responses after Spinal Cord Injury. Cells 2021;10:2783. doi:10.3390/cells10102783.
19. Garcia E, Aguilar-Cevallos J, Silva-Garcia R, et al. Cytokine and Growth Factor Activation In Vivo and In Vitro after Spinal Cord Injury. Mediators Inflamm 2016:9476020 2016. doi:10.1155/2016/9476020.
20. Ankeny DP, Popovich PG. Mechanisms and implications of adaptive immune responses after traumatic spinal cord injury. Neuroscience 2009;158:1112–1121. doi:10.1016/j.neuroscience.2008.07.001.
21. Chacón SC, Nieto-sampedro M. Pathophysiology of spinal cord injury. A review. Vet Méx 2005;36:75–86.
22. Silver J, Miller JH. Regeneration beyond the glial scar. Nat Rev Neurosci 2004;5:146–156. doi:10.1038/nrn1326.
23. Tederko P, Krasuski M, Kiwerski J, et al. Strategies for neuroprotection following spinal cord injury. Ortop Traumatol Rehabil 2009;11:103–110.
24. Khorasanizadeh M, Eskian M, Vaccaro AR, et al. Granulocyte Colony-Stimulating Factor (G-CSF) for the Treatment of Spinal Cord Injury. CNS Drugs 2017;31:911–937. doi:10.1007/s40263-017-0472-6.
25. Cobianchi S, Arbat-Plana A, Lopez-Alvarez VM, et al. Neuroprotective Effects of Exercise Treatments After Injury: The Dual Role of Neurotrophic Factors. Curr Neuropharmacol 2017;15:495–518. doi:10.2174/1570159x14666160330105132.
26. Brodkey JS, Richards DE, Blasingame JP, et al. Reversible spinal cord trauma in cats. Additive effects of direct pressure and ischemia. J Neurosurg 1972;37:591–593. doi:10.3171/jns.1972.37.5.0591.
27. Delamarter RB, Sherman J, Carr JB. Pathophysiology of spinal cord injury. Recovery after immediate and delayed decompression. J Bone Joint Surg Am 1995;77:1042–1049. doi:10.2106/00004623-199507000-00010.
28. Dolan EJ, Tator CH, Endrenyi L. The value of decompression for acute experimental spinal cord compression injury. J Neurosurg 1980;53:749–755). doi:10.3171/jns.1980.53.6.0749.
29. La Rosa G, Conti A, Cardali S, et al. Does early decompression improve the neurological outcome of spinal cord injured patients? Appraisal of the literature using a meta-analytical approach. Spinal Cord 2004;42:503–512. doi:10.1038/sj.sc.3101627.
30. Pivovarova NB, Andrews SB. Calcium-dependent mitochondrial function and dysfunction in neurons. FEBS J 2010;277:3622–3636. doi:10.1111/j.1742-4658.2010.07754.x.
31. Li S, Stys PK. Na(+)-K(+)-ATPase inhibition and depolarization induce glutamate release via reverse Na(+)-dependent transport in spinal cord white matter. Neuroscience 2001;107:675–683. doi:10.1016/s0306-4522(01)00385-2.
32. Stys PK, Waxman SG, Ransom BR. Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na+ channels and Na(+)–Ca2+ exchanger. J Neurosci 1992;12:430–439. doi:10.1523/JNEUROSCI.12-02-00430.1992.
33. Regan RF, Choi DW. Glutamate neurotoxicity in spinal cord cell culture. Neuroscience 1991;43:585–591. doi:10.1016/0306-4522(91)90317-h.
34. Schwartz G, Fehlings MG. Evaluation of the neuroprotective effects of sodium channel blockers after spinal cord injury: improved behavioral and neuroanatomical recovery with riluzole. J Neurosurg 2001;94(2 Suppl):245–256. doi:10.3171/spi.2001.94.2.0245.
35. Kumarasamy D, Viswanathan VK, Shetty AP, et al. The Role of Riluzole in Acute Traumatic Cervical Spinal Cord Injury with Incomplete Neurological Deficit: A Prospective, Randomised Controlled Study. Indian J Orthop 2022;56:2160–2168. doi:10.1007/s43465-022-00758-6.
36. Fehlings MG, Moghaddamjou A, Harrop JS, et al. Safety and Efficacy of Riluzole in Acute Spinal Cord Injury Study (RISCIS): A Multi-Center, Randomized, Placebo-Controlled, Double-Blinded Trial. J Neurotrauma 2023;40:1878–1888. doi:10.1089/neu.2023.0163.
37. Mitochondria Duchen MR. calcium-dependent neuronal death, and neurodegenerative disease. Pflugers Arch 2012;464:111–121. doi:10.1007/s00424-012-1112-0.
38. Xiong Y, Rabchevsky AG, Hall ED. Role of peroxynitrite in secondary oxidative damage after spinal cord injury. J Neurochem 2007;100:639–649. doi:10.1111/j.1471-4159.2006.04312.x.
39. Hall ED, Wang JA, Bosken JM, et al. Lipid peroxidation in brain or spinal cord mitochondria after injury. J Bioenerg Biomembr 2016;48:169–174. doi:10.1007/s10863-015-9600-5.
40. Hall ED, Braughler JM. Glucocorticoid mechanisms in acute spinal cord injury: a review and therapeutic rationale. Surg Neurol 1982;18:320–327. doi:10.1016/0090-3019(82)90140-9.
41. Bains M, Hall ED. Antioxidant therapies in traumatic brain and spinal cord injury. Biochim Biophys Acta 2012;1822:675–684. doi:10.1016/j.bbadis.2011.10.017.
42. Fujimoto T, Nakamura T, Ikeda T, et al. Potent protective effects of melatonin on experimental spinal cord injury. Spine (Phila Pa 1976) 2000;25:769–775. doi:10.1097/00007632-200004010-00003.
43. Sanchez-Barcelo EJ, Rueda N, Mediavilla MD, et al. Clinical Uses of Melatonin in Neurological Diseases and Mental and Behavioural Disorders. Curr Med Chem 2017;24:3851–3878. doi:10.2174/0929867324666170718105557.
44. Schiaveto-de-Souza A, da-Silva CA, Defino HL, et al. Effect of melatonin on the functional recovery from experimental traumatic compression of the spinal cord. Braz J Med Biol Res 2013;46:348–358. doi:10.1590/1414-431x20132322.
45. Diaz-Ruiz A, Rios C, Duarte I, et al. Cyclosporin-A inhibits lipid peroxidation after spinal cord injury in rats. Neurosci Lett 1999;266:61–64. doi:10.1016/s0304-3940(99)00255-4.
46. Ibarra A, Diaz-Ruiz A. Protective effect of cyclosporin-A in spinal cord injury: an overview. Curr Med Chem 2006;13:2703–2710. doi:10.2174/092986706778201503.
47. Robert AA, Zamzami M, Sam AE, et al. The efficacy of antioxidants in functional recovery of spinal cord injured rats: an experimental study. Neurol Sci 2012;33:785–791. doi:10.1007/s10072-011-0829-4.
48. Yan M, Yang M, Shao W, et al. High-dose ascorbic acid administration improves functional recovery in rats with spinal cord

contusion injury. Spinal Cord 2014;52:803–808. doi:10.1038/sc.2014.135.
49. Zadeh-Ardabili PM, Rad SK, Rad SK, et al. Palm vitamin E reduces locomotor dysfunction and morphological changes induced by spinal cord injury and protects against oxidative damage. Sci Rep 2017;7:14365. doi:10.1038/s41598-017-14765-3.
50. Huang WL, King VR, Curran OE, et al. A combination of intravenous and dietary docosahexaenoic acid significantly improves outcome after spinal cord injury. Brain 2007;130:3004–3019. doi:10.1093/brain/awm223.
51. Beattie MS, Farooqui AA, Bresnahan JC. Review of current evidence for apoptosis after spinal cord injury. J Neurotrauma 2000;17:915–925. doi:10.1089/neu.2000.17.915.
52. Liu XZ, Xu XM, Hu R, et al. Neuronal and glial apoptosis after traumatic spinal cord injury. J Neurosci 1997;17:5395–5406. doi:10.1523/JNEUROSCI.17-14-05395.1997.
53. Shi Z, Yuan S, Shi L, et al. Programmed cell death in spinal cord injury pathogenesis and therapy. Cell Prolif 2021;54:e12992. doi:10.1111/cpr.12992.
54. Lu J, Ashwell KW, Waite P. Advances in secondary spinal cord injury: role of apoptosis. Spine (Phila Pa 1976) 2000;25:1859–1866. doi:10.1097/00007632-200007150-00022.
55. Festoff BW, Ameenuddin S, Arnold PM, et al. Minocycline neuroprotects, reduces microgliosis, and inhibits caspase protease expression early after spinal cord injury. J Neurochem 2006;97:1314–1326. doi:10.1111/j.1471-4159.2006.03799.x.
56. Lee SM, Yune TY, Kim SJ, et al. Minocycline reduces cell death and improves functional recovery after traumatic spinal cord injury in rats. J Neurotrauma 2003;20:1017–1027. doi:10.1089/089771503770195867.
57. Zhou KL, Chen DH, Jin HM, et al. Effects of calcitriol on experimental spinal cord injury in rats. Spinal Cord 2016;54:510–516. doi:10.1038/sc.2015.217.
58. Wang C, Liu C, Gao K, et al. Metformin preconditioning provides neuroprotection through enhancement of autophagy and suppression of inflammation and apoptosis after spinal cord injury. Biochem Biophys Res Commun 2016;477:534–540. doi:10.1016/j.bbrc.2016.05.148.
59. Zhang T, Wang F, Li K, et al. Therapeutic effect of metformin on inflammation and apoptosis after spinal cord injury in rats through the Wnt/β-catenin signaling pathway. Neurosci Lett 2020;739:135440. doi:10.1016/j.neulet.2020.135440.
60. Déry MA, Rousseau G, Benderdour M, et al. Atorvastatin prevents early apoptosis after thoracic spinal cord contusion injury and promotes locomotion recovery. Neurosci Lett 2009;453:73–76. doi:10.1016/j.neulet.2009.01.062.
61. Bimbova K, Bacova M, Kisucka A, et al. A Single Dose of Atorvastatin Applied Acutely after Spinal Cord Injury Suppresses Inflammation, Apoptosis, and Promotes Axon Outgrowth, Which Might Be Essential for Favorable Functional Outcome. Int J Mol Sci 2018;19:1106. doi:10.3390/ijms19041106.
62. Gao K, Shen Z, Yuan Y, et al. Simvastatin inhibits neural cell apoptosis and promotes locomotor recovery via activation of the Wnt/β-catenin signaling pathway after spinal cord injury. J Neurochem 2016;138:139–149. doi:10.1111/jnc.13382.
63. Chen C, Yang Q, Ma X. Synergistic effect of ascorbic acid and taurine in the treatment of a spinal cord injury-induced model in rats. 3 Biotech 2020;10:50. doi:10.1007/s13205-019-2032-x.
64. Chen HC, Hsu PW, Tzaan WC, et al. Effects of the combined administration of vitamins C and E on the oxidative stress status and programmed cell death pathways after experimental spinal cord injury. Spinal Cord 2014;52:24–28. doi:10.1038/sc.2013.140.
65. Nishio Y, Koda M, Kamada T, et al. Granulocyte colony-stimulating factor attenuates neuronal death and promotes functional recovery after spinal cord injury in mice. J Neuropathol Exp Neurol 2007;66:724–731. doi:10.1097/nen.0b013e3181257176.
66. Pitzer C, Klussmann S, Krüger C, et al. The hematopoietic factor granulocyte-colony stimulating factor improves outcomes in experimental spinal cord injury. J Neurochem 2010;113:930–942. doi:10.1111/j.1471-4159.2010.06659.x.
67. Derakhshanrad N, Saberi H, Yekaninejad MS, et al. Safety of granulocyte colony-stimulating factor (G-CSF) administration for post-rehabilitated motor complete spinal cord injury patients: an open-label, phase I study. Cell Transplant 2013;22(Suppl 1):S139–S146. doi:10.3727/096368913x672109.
68. Saberi H, Derakhshanrad N, Yekaninejad MS. Comparison of neurological and functional outcomes after administration of granulocyte-colony-stimulating factor in motor-complete versus motor-incomplete postrehabilitated, chronic spinal cord injuries: a phase I/II study. Cell Transplant 2014;23(Suppl 1):S19–S23. doi:10.3727/096368914x684943.
69. Derakhshanrad N, Saberi H, Yekaninejad MS, et al. Granulocyte-colony stimulating factor administration for neurological improvement in patients with postrehabilitation chronic incomplete traumatic spinal cord injuries: a double-blind randomized controlled clinical trial. J Neurosurg Spine 2018;29:97–107. doi:10.3171/2017.11.SPINE17769.
70. Derakhshanrad N, Saberi H, Yekaninejad MS, et al. Subcutaneous granulocyte colony-stimulating factor administration for subacute traumatic spinal cord injuries, report of neurological and functional outcomes: a double-blind randomized controlled clinical trial. J Neurosurg Spine 2018;30:19–30. doi:10.3171/2018.6.SPINE18209.
71. Zhou Y, Zhang H, Zheng B, et al. Retinoic Acid Induced-Autophagic Flux Inhibits ER-Stress Dependent Apoptosis and Prevents Disruption of Blood-Spinal Cord Barrier after Spinal Cord Injury. Int J Biol Sci 2016;12:87–99. doi:10.7150/ijbs.13229.
72. Schnell L, Fearn S, Schwab ME, et al. Cytokine-induced acute inflammation in the brain and spinal cord. J Neuropathol Exp Neurol 1999;58:245–254. doi:10.1097/00005072-199903000-00004.
73. Donnelly DJ, Popovich PG. Inflammation and its role in neuroprotection, axonal regeneration, and functional recovery after spinal cord injury. Exp Neurol 2008;209:378–388. doi:10.1016/j.expneurol.2007.06.009.
74. Fleming JC, Norenberg MD, Ramsay DA, et al. The cellular inflammatory response in human spinal cords after injury. Brain 2006;129:3249–3269. doi:10.1093/brain/awl296.
75. Moghaddam A, Child C, Bruckner T, et al. Posttraumatic inflammation as a key to neuroregeneration after traumatic spinal cord injury. Int J Mol Sci 2015;16:7900–7916. doi:10.3390/ijms16047900.
76. Ahuja CS, Fehlings M. Concise Review: Bridging the Gap: Novel Neuroregenerative and Neuroprotective Strategies in Spinal Cord Injury. Stem Cells Transl Med 2016;5:914–924. doi:10.5966/sctm.2015-0381.
77. 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 1990;322:1405–1411. doi:10.1056/NEJM199005173222001.
78. García E, Silva-García R, Flores-Romero A, et al. The Severity of Spinal Cord Injury Determines the Inflammatory Gene Expression Pattern after Immunization with Neural-Derived Peptides. J Mol Neurosci 2018;65:190–195. doi:10.1007/s12031-018-1077-3.
79. Garcia E, Buzoianu-Anguiano V, Silva-Garcia R, et al. Use of Cells, Supplements, and Peptides as Therapeutic Strategies for Modulating Inflammation after Spinal Cord Injury: An Update. Int J Mol Sci 2023;24:13946. doi:10.3390/ijms241813946.
80. Rodríguez-Barrera R, Flores-Romero A, García E, et al. Immunization with neural-derived peptides increases neurogenesis in rats with chronic spinal cord injury. CNS Neurosci Ther 2020;26:650–658. doi:10.1111/cns.13368.
81. Manzhulo I, Tyrtyshnaia A, Kipryushina Y, et al. Docosahexaenoic acid improves motor function in the model of spinal cord

injury. Neurosci Lett 2018;672:6–14. doi:10.1016/j.neulet.2018.02.028.
82. Li D, Tian H, Li X, et al. Zinc promotes functional recovery after spinal cord injury by activating Nrf2/HO-1 defense pathway and inhibiting inflammation of NLRP3 in nerve cells. Life Sci 2020;245:117351. doi:10.1016/j.lfs.2020.117351.
83. Di Paola R, Impellizzeri D, Salinaro AT, et al. Administration of carnosine in the treatment of acute spinal cord injury. Biochem Pharmacol 2011;82:1478–1489. doi:10.1016/j.bcp.2011.07.074.
84. Kigerl KA, Hall JC, Wang L, et al. Gut dysbiosis impairs recovery after spinal cord injury. J Exp Med 2016;213:2603–2620. doi:10.1084/jem.20151345.
85. Jing Y, Yu Y, Bai F, et al. Effect of fecal microbiota transplantation on neurological restoration in a spinal cord injury mouse model: involvement of brain-gut axis. Microbiome 2021;9:59. doi:10.1186/s40168-021-01007-y.
86. Abrams MB, Nilsson I, Lewandowski SA, et al. Imatinib enhances functional outcome after spinal cord injury. PLoS One 2012;7:e38760. doi:10.1371/journal.pone.0038760.
87. Pannu R, Christie DK, Barbosa E, et al. Post-trauma Lipitor treatment prevents endothelial dysfunction, facilitates neuroprotection, and promotes locomotor recovery following spinal cord injury. J Neurochem 2007;101:182–200. doi:10.1111/j.1471-4159.2006.04354.x.
88. Afshari K, Dehdashtian A, Haddadi NS, et al. Anti-inflammatory effects of Metformin improve the neuropathic pain and locomotor activity in spinal cord injured rats: introduction of an alternative therapy. Spinal Cord 2018;56:1032–1041. doi:10.1038/s41393-018-0168-x.
89. Liu T, Zhu W, Zhang X, et al. Recent Advances in Cell and Functional Biomaterial Treatment for Spinal Cord Injury. Biomed Res Int 2022;2022:5079153. doi:10.1155/2022/5079153.
90. Wiliams RR, Bunge MB. Schwann cell transplantation: a repair strategy for spinal cord injury? Prog Brain Res 2012;201:295–312. doi:10.1016/B978-0-444-59544-7.00014-7.
91. Kanno H, Pearse DD, Ozawa H, et al. Schwann cell transplantation for spinal cord injury repair: its significant therapeutic potential and prospectus. Rev Neurosci 2015;26:121–128. doi:10.1515/revneuro-2014-0068.
92. Zhou XH, Ning GZ, Feng SQ, et al. Transplantation of autologous activated Schwann cells in the treatment of spinal cord injury: six cases, more than five years of follow-up. Cell Transplant 2012;21(Suppl 1):S39–S47. doi:10.3727/096368912x633752.
93. Gant KL, Guest JD, Palermo AE, et al. Phase 1 Safety Trial of Autologous Human Schwann Cell Transplantation in Chronic Spinal Cord Injury. J Neurotrauma 2022;39:285–299. doi:10.1089/neu.2020.7590.
94. Dasari VR, Veeravalli KK, Dinh DH. Mesenchymal stem cells in the treatment of spinal cord injuries: A review. World J Stem Cells 2014;6:120–133. doi:10.4252/wjsc.v6.i2.120.
95. Quertainmont R, Cantinieaux D, Botman O, et al. Mesenchymal stem cell graft improves recovery after spinal cord injury in adult rats through neurotrophic and pro-angiogenic actions. PLoS One 2012;7:e39500. doi:10.1371/journal.pone.0039500.
96. Sasaki M, Radtke C, Tan AM, et al. BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. J Neurosci 2009;29:14932–14941. doi:10.1523/JNEUROSCI.2769-09.2009.
97. Nakajima H, Uchida K, Guerrero AR, et al. Transplantation of mesenchymal stem cells promotes an alternative pathway of macrophage activation and functional recovery after spinal cord injury. J Neurotrauma 2012;29:1614–1625. doi:10.1089/neu.2011.2109.
98. Hammadi AA, Marino A, Farhan S. Clinical response of 277 patients with spinal cord injury to stem cell therapy in Iraq. Int J Stem Cells 2012;5:76–78. doi:10.15283/ijsc.2012.5.1.76.
99. Kishk NA, Gabr H, Hamdy S, et al. Case-control series of intrathecal autologous bone marrow mesenchymal stem cell therapy for chronic spinal cord injury. Neurorehabil Neural Repair 2010;24:702–708. doi:10.1177/1545968310369801.
100. Nakhjavan-Shahraki B, Yousefifard M, Rahimi-Movaghar V, et al. Transplantation of olfactory ensheathing cells on functional recovery and neuropathic pain after spinal cord injury; systematic review and meta-analysis. Sci Rep 2018;8:325. doi:10.1038/s41598-017-18754-4.
101. Yao R, Murtaza M, Velasquez JT, et al. Olfactory Ensheathing Cells for Spinal Cord Injury: Sniffing Out the Issues. Cell Transplant 2018;27:879–889. doi:10.1177/0963689718779353.
102. Ursavas S, Darici H, Karaoz E. Olfactory ensheathing cells: Unique glial cells promising for treatments of spinal cord injury. J Neurosci Res 2021;99:1579–1597. doi:10.1002/jnr.24817.
103. Assinck P, Duncan GJ, Hilton BJ, Plemel JR, Tetzlaff W. Cell transplantation therapy for spinal cord injury. Nat Neurosci 2017;20:637–647. doi:10.1038/nn.4541.
104. de Freria CM, Van Niekerk E, Blesch A, et al. Neural Stem Cells: Promoting Axonal Regeneration and Spinal Cord Connectivity. Cells 2021;10:3296. doi:10.3390/cells10123296.
105. Rong Y, Liu W, Wang J, et al. Neural stem cell-derived small extracellular vesicles attenuate apoptosis and neuroinflammation after traumatic spinal cord injury by activating autophagy. Cell Death Dis 2019;10:340. doi:10.1038/s41419-019-1571-8.
106. Ceto S, Sekiguchi KJ, Takashima Y, et al. Neural Stem Cell Grafts Form Extensive Synaptic Networks that Integrate with Host Circuits after Spinal Cord Injury. Cell Stem Cell 2020;27:430–440.e5. doi:10.1016/j.stem.2020.07.007.
107. Shin JC, Kim KN, Yoo J, et al. Clinical Trial of Human Fetal Brain-Derived Neural Stem/Progenitor Cell Transplantation in Patients with Traumatic Cervical Spinal Cord Injury. Neural Plast 2015:630932 2015. doi:10.1155/2015/630932.
108. Levi AD, Okonkwo DO, Park P, et al. Emerging Safety of Intramedullary Transplantation of Human Neural Stem Cells in Chronic Cervical and Thoracic Spinal Cord Injury. Neurosurgery 2018;82:562–575. doi:10.1093/neuros/nyx250.
109. Curtis E, Martin JR, Gabel B, et al. A First-in-Human, Phase I Study of Neural Stem Cell Transplantation for Chronic Spinal Cord Injury. Cell Stem Cell 2018;22:941–950.e6. doi:10.1016/j.stem.2018.05.014.
110. Levi AD, Anderson KD, Okonkwo DO, et al. Clinical Outcomes from a Multi-Center Study of Human Neural Stem Cell Transplantation in Chronic Cervical Spinal Cord Injury. J Neurotrauma 2019;36:891–902. doi:10.1089/neu.2018.5843.
111. Hodgetts SI, Edel M, Harvey AR. The State of Play with iPSCs and Spinal Cord Injury Models. J Clin Med 2015;4:193–203. doi:10.3390/jcm4010193.
112. Saitoh SS, Tanabe S, Muramatsu R. Circulating factors that influence the central nervous system remyelination. Curr Opin Pharmacol 2022;62:130–136. doi:10.1016/j.coph.2021.12.001.
113. Thuret S, Moon LD, Gage FH. Therapeutic interventions after spinal cord injury [published correction appears in Nat Rev Neurosci 2006;7:628–643. doi:10.1038/nrn1955.
114. Liebscher T, Schnell L, Schnell D, et al. Nogo-A antibody improves regeneration and locomotion of spinal cord-injured rats. Ann Neurol 2005;58:706–719. doi:10.1002/ana.20627.
115. Freund P, Schmidlin E, Wannier T, et al. Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates [published correction appears in Nat Med 2006;12:790–792. doi:10.1038/nm1436.
116. Wang YT, Lu XM, Chen KT, et al. Immunotherapy strategies for spinal cord injury. Curr Pharm Biotechnol 2015;16:492–505. doi:10.2174/1389201016061504071126 46.

117. Lu XM, Wei JX, Xiao L, et al. Experimental and Clinical Advances in Immunotherapy Strategies for Spinal Cord Injury Target on MAIs and Their Receptors. Curr Pharm Des 2016;22:728–737. doi:10.2174/1381612822666151204000855.
118. Guercio JR, Kralic JE, Marrotte EJ, et al. Spinal cord injury pharmacotherapy: Current research & development and competitive commercial landscape as of 2015. J Spinal Cord Med 2019;42:102–122. doi:10.1080/10790268.2018.1439803.
119. Fehlings MG, Kim KD, Aarabi B, et al. Rho Inhibitor VX-210 in Acute Traumatic Subaxial Cervical Spinal Cord Injury: Design of the SPinal Cord Injury Rho INhibition InvestiGation (SPRING) Clinical Trial. J Neurotrauma 2018;35:1049–1056. doi:10.1089/neu.2017.5434.
120. Fehlings MG, Chen Y, Aarabi B, et al. A Randomized Controlled Trial of Local Delivery of a Rho Inhibitor (VX-210) in Patients with Acute Traumatic Cervical Spinal Cord Injury. J Neurotrauma 2021;38:2065–2072. doi:10.1089/neu.2020.7096.
121. Uyeda A, Muramatsu R. Molecular Mechanisms of Central Nervous System Axonal Regeneration and Remyelination: A Review. Int J Mol Sci 2020;21:8116. doi:10.3390/ijms21218116.
122. Deshmukh VA, Tardif V, Lyssiotis CA, et al. A regenerative approach to the treatment of multiple sclerosis. Nature 2013;502:327–332. doi:10.1038/nature12647.
123. Kawabata S, Takano M, Numasawa-Kuroiwa Y, et al. Grafted Human iPS Cell-Derived Oligodendrocyte Precursor Cells Contribute to Robust Remyelination of Demyelinated Axons after Spinal Cord Injury. Stem Cell Reports 2016;6:1–8. doi:10.1016/j.stemcr.2015.11.013.
124. Fessler RG, Ehsanian R, Liu CY, et al. A phase 1/2a dose-escalation study of oligodendrocyte progenitor cells in individuals with subacute cervical spinal cord injury. J Neurosurg Spine 2022;37:812–820 Published 2022 Jul 8. doi:10.3171/2022.5.SPINE22167.
125. Harvey LA. Physiotherapy rehabilitation for people with spinal cord injuries. J Physiother 2016;62:4–11. doi:10.1016/j.jphys.2015.11.004.
126. Sandrow-Feinberg HR, Houlé JD. Exercise after spinal cord injury as an agent for neuroprotection, regeneration, and rehabilitation. Brain Res 2015;1619:12–21. doi:10.1016/j.brainres.2015.03.052.
127. Sachdeva R, Theisen CC, Ninan V, et al. Exercise dependent increase in axon regeneration into peripheral nerve grafts by propriospinal but not sensory neurons after spinal cord injury is associated with modulation of regeneration-associated genes. Exp Neurol 2016;276:72–82. doi:10.1016/j.expneurol.2015.09.004.
128. Huang Z, Wang W, Ma J, et al. mTOR signaling pathway differently regulates central and peripheral axon regeneration. Acta Biochim Biophys Sin (Shanghai) 2017;49:689–695. doi:10.1093/abbs/gmx068.
129. Hogan MK, Hamilton GF, Horner PJ. Neural Stimulation and Molecular Mechanisms of Plasticity and Regeneration: A Review. Front Cell Neurosci 2020;14:271. doi:10.3389/fncel.2020.00271.
130. Bose PK, Hou J, Parmer R, et al. Altered patterns of reflex excitability, balance, and locomotion following spinal cord injury and locomotor training. Front Physiol 2012;3:258. doi:10.3389/fphys.2012.00258.
131. Rahayu UB, Wibowo S, Setyopranoto I, et al. Effectiveness of physiotherapy interventions in brain plasticity, balance and functional ability in stroke survivors: A randomized controlled trial. NeuroRehabilitation 2020;47:463–470. doi:10.3233/NRE-203210.
132. Liu Y, Yin JH, Lee JT, et al. Early Rehabilitation after Acute Stroke: The Golden Recovery Period. Acta Neurol Taiwan 2022.
133. Khan F, Amatya B, Galea MP, et al. Neurorehabilitation: applied neuroplasticity. J Neurol 2017;264:603–615. doi:10.1007/s00415-016-8307-9.
134. Thomas AM, Seidlits SK, Goodman AG, et al. Sonic hedgehog and neurotrophin-3 increase oligodendrocyte numbers and myelination after spinal cord injury. Integr Biol (Camb) 2014;6:694–705. doi:10.1039/c4ib00009a.
135. Vismara I, Papa S, Rossi F, et al. Current Options for Cell Therapy in Spinal Cord Injury. Trends Mol Med 2017;23:831–849. doi:10.1016/j.molmed.2017.07.005.
136. Ziemba AM, Gilbert RJ. Biomaterials for Local, Controlled Drug Delivery to the Injured Spinal Cord. Front Pharmacol 2017;8:245. doi:10.3389/fphar.2017.00245.
137. Ashammakhi N, Kim HJ, Ehsanipour A, et al. Regenerative Therapies for Spinal Cord Injury. Tissue Eng Part B Rev 2019;25:471–491. doi:10.1089/ten.TEB.2019.0182.
138. Führmann T, Anandakumaran PN, Shoichet MS. Combinatorial Therapies After Spinal Cord Injury: How Can Biomaterials Help? Adv Healthc Mater 2017;6. doi:10.1002/adhm.201601130.
139. Boisserand LS, Kodama T, Papassin J, et al. Biomaterial Applications in Cell-Based Therapy in Experimental Stroke. Stem Cells Int 2016:6810562 2016. doi:10.1155/2016/6810562.
140. Haggerty AE, Oudega M. Biomaterials for spinal cord repair. Neurosci Bull 2013;29:445–459. doi:10.1007/s12264-013-1362-7.
141. Kong XB, Tang QY, Chen XY, et al. Polyethylene glycol as a promising synthetic material for the repair of spinal cord injury. Neural Regen Res 2017;12:1003–1008. doi:10.4103/1673-5374.208597.
142. Lu X, Perera TH, Aria AB, et al. Polyethylene glycol in spinal cord injury repair: a critical review. J Exp Pharmacol 2018;10:37–49. doi:10.2147/JEP.S148944.
143. Brazda N, Estrada V, Voss C, et al. Experimental Strategies to Bridge Large Tissue Gaps in the Injured Spinal Cord after Acute and Chronic Lesion. J Vis Exp 2016(110):e53331. doi:10.3791/53331.
144. Dumont CM, Carlson MA, Munsell MK, et al. Aligned hydrogel tubes guide regeneration following spinal cord injury. Acta Biomater 2019;86:312–322. doi:10.1016/j.actbio.2018.12.052.
145. Li HY, Führmann T, Zhou Y, et al. Host reaction to poly(2-hydroxyethyl methacrylate) scaffolds in a small spinal cord injury model. J Mater Sci Mater Med 2013;24:2001–2011. doi:10.1007/s10856-013-4956-8.
146. Biological evaluation of medical devices. Part 1: Evaluation and testing within a risk management process. International Standard ISO 10993-1 Fifth Ed 2018:41 2018-08.
147. Afshari K, Momeni Roudsari N, Lashgari NA, et al. Antibiotics with therapeutic effects on spinal cord injury: a review. Fundam Clin Pharmacol 2021;35:277–304. doi:10.1111/fcp.12605.
148. Diaz-Ruiz A, Nader-Kawachi J, Calderón-Estrella F, et al. Dapsone, More than an Effective Neuro and Cytoprotective Drug. Curr Neuropharmacol 2022;20:194–210. doi:10.2174/1570159x19666210617143108.
149. Sharma A, Sharma HS. Monoclonal antibodies as novel neurotherapeutic agents in CNS injury and repair. Int Rev Neurobiol 2012;102:23–45. doi:10.1016/B978-0-12-386986-9.00002-8.
150. Al Mamun A, Monalisa I, Tul Kubra K, et al. Advances in immunotherapy for the treatment of spinal cord injury. Immunobiology 2021;226:152033. doi:10.1016/j.imbio.2020.152033.
151. Zhen-Gang L, Fan Y, Jingwei S, et al. Revisiting the immune landscape post spinal cord injury: More than black and white. Front Aging Neurosci 2022;14:963539. doi:10.3389/fnagi.2022.963539.
152. Askarifirouzjaei H, Khajoueinejad L, Salek Farrokhi A, et al. Implications of immunotherapy with high-dose glatiramer acetate in acute phase of spinal cord injury in rats. Immunopharmacol Immunotoxicol 2019;41:150–162. doi:10.1080/08923973.2019.1566362.
153. Mothe AJ, Jacobson PB, Caprelli M, et al. Delayed administration of elezanumab, a human anti-RGMa neutralizing monoclonal antibody, promotes recovery following cervical spinal cord injury. Neurobiol Dis 2022;172:105812. doi:10.1016/j.nbd.2022.105812.
154. Rodríguez-Barrera R, Fernández-Presas AM, García E, et al. Immunization with a neural-derived peptide protects the spinal cord from

apoptosis after traumatic injury. Biomed Res Int 2013:827517 2013. doi:10.1155/2013/827517.
155. Martiñón S, García-Vences E, Toscano-Tejeida D, et al. Long-term production of BDNF and NT-3 induced by A91-immunization after spinal cord injury. BMC Neurosci 2016;17:42. doi:10.1186/s12868-016-0267-6.
156. Bermeo G, Ibarra A, García E, et al. Monocyte locomotion inhibitory factor produced by E. histolytica improves motor recovery and develops neuroprotection after traumatic injury to the spinal cord. Biomed Res Int 2013:340727 2013. doi:10.1155/2013/340727.
157. Edwards DJ, Forrest G, Cortes M, et al. Walking improvement in chronic incomplete spinal cord injury with exoskeleton robotic training (WISE): a randomized controlled trial. Spinal Cord 2022;60:522–532. doi:10.1038/s41393-022-00751-8.
158. Corbianco S, Cavallini G, Dini M, et al. Energy cost and psychological impact of robotic-assisted gait training in people with spinal cord injury: effect of two different types of devices. Neurol Sci 2021;42:3357–3366. doi:10.1007/s10072-020-04954-w.
159. Singh H, Unger J, Zariffa J, et al. Robot-assisted upper extremity rehabilitation for cervical spinal cord injuries: a systematic scoping review. Disabil Rehabil Assist Technol 2018;13:704–715. doi:10.1080/17483107.2018.1425747.
160. Morone G, de Sire A, Martino Cinnera A, et al. Upper Limb Robotic Rehabilitation for Patients with Cervical Spinal Cord Injury: A Comprehensive Review. Brain Sci 2021;11:1630. doi:10.3390/brainsci11121630.
161. Vukojevic J, Milavić M, Perović D, et al. Pentadecapeptide BPC 157 and the central nervous system. Neural Regen Res 2022;17:482–487. doi:10.4103/1673-5374.320969.
162. Perovic D, Milavic M, Dokuzovic S, et al. Novel Therapeutic Effects in Rat Spinal Cord Injuries: Recovery of the Definitive and Early Spinal Cord Injury by the Administration of Pentadecapeptide BPC 157 Therapy. Curr Issues Mol Biol 2022;44:1901–1927. doi:10.3390/cimb44050130.
163. Joaquim AF, Daniel JW, Schroeder GD, et al. Neuroprotective Agents as an Adjuvant Treatment in Patients with Acute Spinal Cord Injuries: A Qualitative Systematic Review of Randomized Trials. Clin Spine Surg 2020;33:65–75. doi:10.1097/BSD.0000000000000861.
164. Cao Y, Zhu S, Yu B, et al. Single-cell RNA sequencing for traumatic spinal cord injury. FASEB J 2022;36:e22656. doi:10.1096/fj.202200943R.