2022

Oxidative stress cascades in spinal cord injury and therapeutic intervention development

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

OXIDATIVE STRESS CASCADES IN SPINAL CORD INJURY AND THERAPEUTIC INTERVENTION DEVELOPMENT

by

CHRISTIAN ALEXANDER ARBELAEZ

B.S., Boston College, 2020

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2022

Approved by

First Reader ____

Jean-Jacques Soghomonian, Ph.D.
Associate Professor of Anatomy and Neurobiology

Second Reader ____

Yang D. Teng, Ph.D., M.S. M.D.
Professor of PM&R, Harvard Medical School, Boston, MA

DEDICATION

I would like to dedicate this thesis in the memory of Professor Neil Wolfman (1953-2020). Professor Wolfman was my first mentor at Boston College and was someone who always believed in my abilities to reach my academic and professional goals. Whenever I was in doubt of who I was, or what I could be, he provided the advice and wisdom to remind me that I am capable of anything I set my mind to. This work is both a testimony to his belief in me and the power that mentors can have in inspiring students to achieve their dreams. Thank you, Professor Wolfman.

.

ACKNOWLEDGMENTS

This thesis was greatly supported by my thesis mentor, Prof. Yang D. Teng, who promoted my capacity of scientific reasoning, research skills, academic presentation, and biomedical professionalism throughout this thesis research training process at his laboratory. Prof. Teng and Prof. Soghomonian who was the second reader of the thesis both also provided important proofreading and detailed editing for the completion of this final copy of the thesis. Additionally, the support from my loving girlfriend Katie, best friends from Boston College, my family, and peers from Boston University were influential for the successful completion of my training project.

OXIDATIVE STRESS CASCADES IN SPINAL CORD INJURY AND THERAPEUTIC INTERVENTION DEVELOPMENT

CHRISTIAN ALEXANDER ARBELAEZ

ABSTRACT

Secondary injury (SI) after spinal cord trauma is characterized by the multiple pathophysiological events that are set in motion by a primary mechanical insult to the spinal cord. SI produces a post-spinal cord injury (SCI) microenvironment that severely limits recovery, and results in more functional deficits. Many advances have been made to increase the scientific understanding of SI mechanisms, but there remains a lack of standardized clinical treatment to mitigate the damage caused by secondary pathophysiology. The classification of SI processes can be done based on the biological systems they take place: vascular (hemorrhagic necrosis, ischemia, edema), immune (cytokine and hormone release, residential inflammatory cells, peripheral inflammatory and immune cells), and neuronal/glial systems (excitotoxicity, mitochondrial dysfunction, ionic disturbances, overproduction of reactive oxygen and nitrogen species, cell death pathway activation, and axon degeneration). Regarding mechanisms underlying the SI processes (i.e., SI mechanisms: the biochemical events that exacerbate structural and functional losses), SCI-induced elevation of reactive oxygen and reactive nitrogen species (ROS/RNS) serve as one of the most potent triggers of neural cell and neurite death and inflammation, essentially affecting all aforementioned systems to worsen neurological dysfunction. In

addition to the role in pathophysiological circumstances, ROS/RNS are also important players in mediating physiological functions (e.g., cell signaling). This feature requires that development of therapeutics for managing post-SCI ROS and RNS must specifically impede the detrimental effects of the radicals in propagating the SI scale without interfering with their physiological roles. In the context of SCI, regulation of ROS/RNS species at their homeostatic levels may serve as an effective therapeutic target for future clinical studies to mitigate neurodegeneration and neuroinflammation, and to promote a permissive environment for endogenous recovery. Based on these facts, Hydrogen Peroxide and Peroxynitrite (a representative ROS and RNS, respectively) were evaluated as SI mediators after SCI and for their potential to be targeted to develop interventions to treat SCI. Overall, the reviewed research findings suggested that oxidative damage cascades should be further studied in laboratory and clinical settings to advance understanding of SI processes of SCI for devising therapies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADP .... Adenosine Diphosphate

Akt .... Protein Kinase B

ATP .... Adenosine Triphosphate

CT .... Computerized Tomography

eIF2α .... Eukaryotic Translation Initiation Factor 2A

ERK .... Extracellular Signal-Regulated Kinase

GSNO .... S-nitrosoglutathione

HDAC1 .... Histone Deacetylase 1

hNSC .... Human Neural Stem Cell

ICAM-1 .... Intercellular Adhesion Molecule 1

IKK .... IκB Kinase

IκB .... Inhibitor of Kappa B

MAPK .... Mitogen-Activated Protein Kinase

MnTBAP .... Manganese (III) Tetrakis (4-benzoic acid) Porphyrin

mPTP .... Mitochondrial Permeability Transition Pore

MP .... Methylprednisolone

MRI .... Magnetic Resonance Imaging

NF-κB .... Nuclear Factor Kappa B

NIK .... NF-κB-Inducing Kinase

NLRP3 .... NOD-, LRR- and Pyrin Domain-Containing Protein 3

PERK .... Protein Kinase R-like Endoplasmic Reticulum Kinase

PI3K.. Phosphoinositide 3-kinases

PLGA...Poly-lactic-*co*-glycolic Acid

PTEN..Phosphatase and Tensin Homolog

RPE..Retinal Pigment Epithelium

SFK..Src Family Kinase

siRNA .... Small Interfering Ribonucleic Acid

INTRODUCTION

Spinal cord injury (SCI) still remains one of the most debilitating neural lesions worldwide, with an estimated 250,000 to 500,000 new cases of SCI occurring every year.[1] In the United States alone there are approximately 17,900 individuals who sustain new SCIs each year and ~288,000 individuals who are currently living with SCI.[2] The presentation of these cases to healthcare centers can either be via traumatic injury (e.g., motor vehicle accident, fall, physical violence, sports-related injury, or other types of injury) or non-traumatic injury (e.g., tumor, infection, or degenerative disc disease).[3] Traumatic SCI accounts for most of the reported cases in the United States and will be the main focus for the rest of this paper.[4]

There are several key demographic trends for traumatic SCI including gender and age. Males account for most of the traumatic SCI patient population in the United States at 78%, while females comprise the remaining 22%. This disparity reflects behavioral differences between genders, as men are more likely to be affected by physical violence (e.g., gunshot wounds) and/or engage in risk-taking activities (e.g., motor vehicle crashes and diving injuries) compared to women as found in a study by Chen et. al. 2013.[4] The distribution of SCI for age is bimodal, with one large peak occurring in individuals aged 15-29 and another large peak in individuals >50. The two peaks are reflective of age-specific risk factors, as the frequency of motor vehicle accidents and high-impact sports injuries are higher in the younger age cohort, whereas the frequency of low-impact falls are higher in the older age cohort.[5] There has also been an increase in average age at injury from 28.7 years old in the 1970s to 43 years old in the 2010s due to a higher SCI incidence rate in the elderly as compared to the general U.S. population (i.e., age 18-64 years, 49.9 new cases per million vs age ≥ 65 years, 87.7 new cases per million in 2009).[2,6] Contributing to the increase in elderly SCI are many risk factors related to aging (e.g., decline in both muscle mass and musculoskeletal strength) that lead to higher susceptibility of experiencing an accidental traumatic injury and the aging U.S. population over the last 50 years.[7]

The major determining factors in the mortality and morbidity of SCI are the neurological level and completeness of the injury. Physical examination of dermatomes (area of the skin innervated by the sensory axons within the segmental peripheral nerve) and myotomes (collection of muscle fibers innervated by the motor axons within the segmental peripheral nerve) by clinicians allows determination of the neurological level and completeness of a particular injury.[8] The neurological level of injury is designated by the most caudal segment of the spinal cord that retains intact sensory (i.e., sensation to light touch and/or pin-prick input to the corresponding dermatome) and motor function (a detectable muscle contraction at grade ≥3 within the corresponding myotome; see reference for grade characterization).[8] In contrast, the anatomical level of injury is defined by the segment of the spinal cord at which the primary damage occurred. The neurological level is the more important parameter to consider clinically, as the secondary injury (SI) spreads above and below the lesion epicenter which can result in an extension of functional impairment in the spinal cord beyond what would be classified by the original anatomical level of the primary injury.[8] The completeness of injury is clinically defined by degree of the sensorimotor loss below the neurological level of injury.[9] Complete injuries are characterized by losing all sensorimotor function below the neurological level of the lesion, whereas individuals with incomplete injuries retain some sensorimotor function below the level.[9] The higher a neurological injury is, the more severe the neurological deficits are, due to greater scales of sensorimotor function loss (e.g., a complete C2 injury results in quadriplegia versus paraplegic sequelae caused by T11 injuries).

Patients with SCI, especially those with higher neurological levels of injury, experience significant alterations to their physical, mental, economical, and social well-being following their injury. Individuals with SCI often require caregiver support, have a reduced life expectancy compared to the general population due to increased risk of health complications, and require substantial financial support for their continued health management post-injury.[1,2,9] SCI-related health complications include autonomic dysreflexia (in injuries occurring above T6), orthostatic hypotension, urinary tract infections, pressure sores, neuropathic pain, gastrointestinal dysfunction, and increased risk of pulmonary embolus, pneumonia, obesity, diabetes, respiratory deficits, and cardiovascular disease.[1,9,10,11] Given the severity of the condition, there have been many efforts to develop therapies to treat major complications in the acute and chronic stages of injury. The historical context of SCI thus provides important insights into the progress of the scientific and medical understanding of this condition, as well as the efforts to develop suitable treatments.

Brief History of Spinal Cord Injury

The first known documentation of spinal cord injury and traumatic brain injury (TBI) came from an Egyptian surgical papyrus written in 1600 BCE. This report highlighted 46 cases of traumatic injury to the head, neck, and chest, which included 6 extensively documented cases of SCI. It is evident through analysis of the documentation that the author of the papyrus had, for that time period, a general understanding of anatomical, surgical, and scientific knowledge, demonstrated by the inclusion of specific complications post-SCI (e.g., loss of sensation and bladder control). Of the 6 selected cases, 2 sustained direct damage to their spinal cords, one suffering from a dislocated vertebra and the other suffering from a crushed vertebra. For these individuals, it was stated that their condition was “an ailment not to be treated”, due to the severity of the injury, limited understanding of the pathophysiological sequelae of SCI, and lack of medical resources (i.e., advanced skills, technology and facility). [12,13]

Therapeutic nihilism regarding SCI continued in the clinical landscape until major medical advancements in the fields of hematology (blood typing), imaging (X-ray, CT, MRI), and surgery (tools and techniques) in the 19th and 20th centuries allowed for safer and more practical general surgical treatment.[11,12]

[14,15,16,17,18,19,20] Dr. Paul Harrington's methodology for improved surgical spinal stabilization in the 1960s was of notable importance, allowing improved alignment and stabilization of the spine for patients with scoliosis using the first implantable spinal instrumentation system. This standard treatment was quickly adopted for the stabilization of spine fractures and dislocations, given their practicality and ease of translation.[20] These medical innovations improved methods for pre-operative planning and operative management and marked new progresses in outcomes post-injury.[12]

In the early 1900s, while some of these clinical advancements in SCI treatment were being made, Dr. Alfred R. Allen, an American neuropathologist and researcher at the University of Pennsylvania, postulated the existence of "secondary" injury mechanisms that were causing significant damage following traumatic SCI.[12] These findings offered another layer to understanding the pathophysiology of neurotrauma extending beyond the mechanical insults.[21] These insults, now defined as the "primary injury", are characterized by their type, strength and speed (e.g., laceration, contusion, or compression) to a particular locus and level of the spinal cord.[22] Dr. Allen's experiments hypothesized that, following the primary injury, there was continuous tissue damage as a result of "edematous and hemorrhagic outpouring into the cord tissue" that increased pressure and chemical activity and contributed to the pathological destruction of the spinal cord.[21,23] He further identified the presence of hemorrhage and edema as damaging SI processes by demonstrating that decompression surgery (laminectomy and myelotomy) to remove these agents from the spinal cord improved neural outcomes in dogs with experimentally-induced contusion SCIs.[12,23] Dr. Allen thus introduced a new pathophysiological understanding of SCI by describing a correlation between the presence of hemorrhage and edema with the extent of the damage observed post-injury.[3,12] Since his work, laboratory and clinical researchers have uncovered numerous additional pathophysiological events following trauma to the spinal cord, categorized by vascular disruptions (i.e., breakdown of the blood-spinal cord barrier), immune and inflammatory response overactivation, neuronal/axonal/dendritic damages, metabolic dysregulation, ionic disturbances, cell death, and oxidative cascades (Figure 1).[12,24,25,26,27,28,29,30] Experimental treatments have aimed to mitigate these secondary pathophysiological events given their roles in exacerbating tissue loss after SCI.[31] This line of research, reasoning and findings helped to initiate the clinical trials for Methylprednisolone, one of the most notable developments of pharmacological interventions in the history of SCI treatment.[32,33]

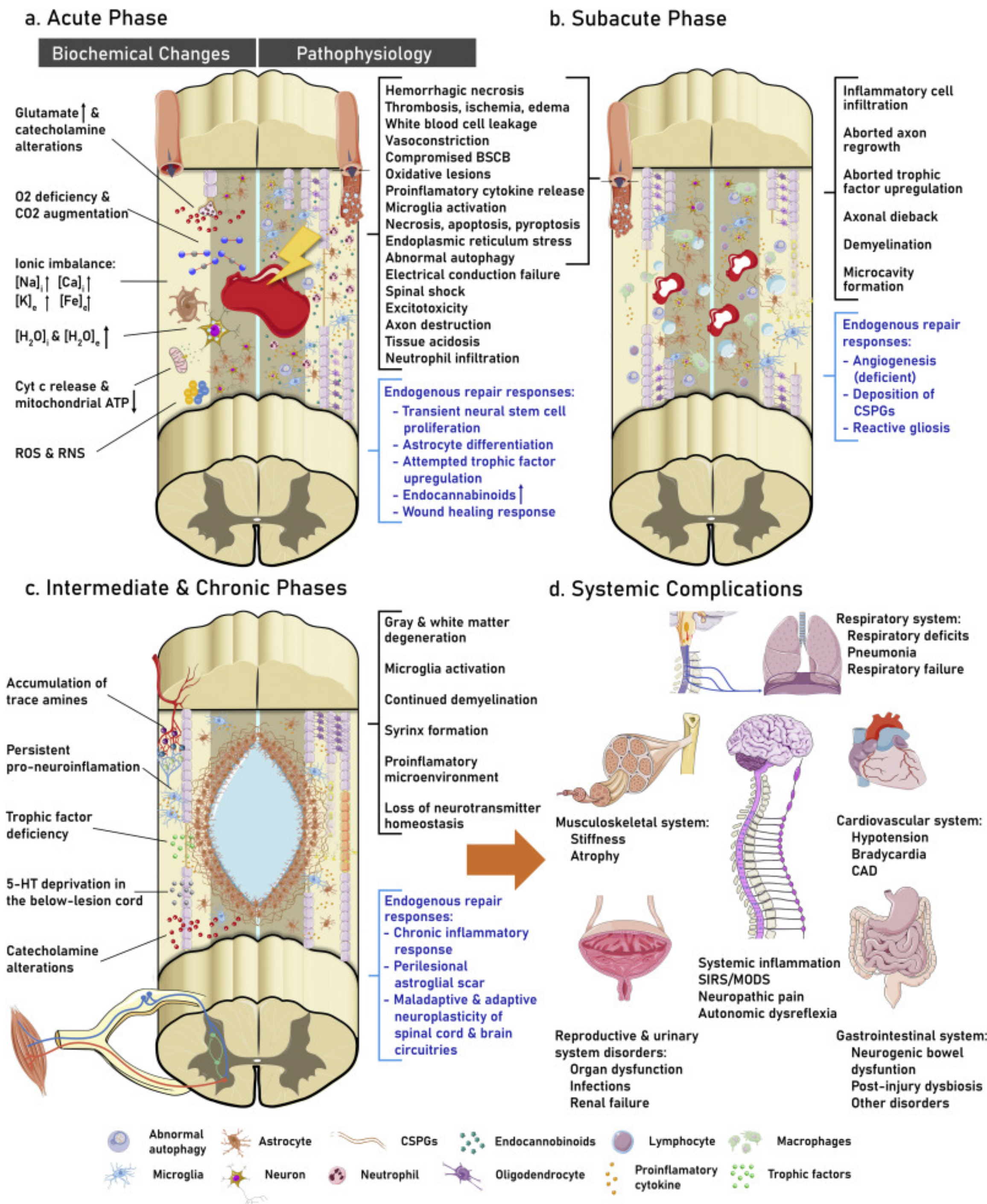

Figure 1. Overview of Secondary Injury Mechanisms.
Illustrative diagram highlighting the biochemical, pathophysiological, and systemic events that occur following spinal cord injury. a) The acute phase of spinal cord injury occurring in the first 0-48 hours after mechanical insult, characterized by damaged central nervous system cells (i.e., neurons,

oligodendrocytes, blood vessels, and axons). Playing major roles in the acute phase of injury are vascular disruptions (hemorrhagic necrosis, thrombosis, ischemia, and edema), immune response (inflammatory cell infiltration and activation), nervous system disruptions (excitotoxicity and conduction failure), and cellular deficits (increased levels of reactive oxygen species/reactive nitrogen species, ionic imbalance, and mitochondrial disruptions) that all contribute to the sequelae of secondary injury. b) The continued presence of these mechanisms during the subacute phase in the ensuing 2-14 days leads to a compounding damage via a continued inflammatory response, ischemia, and increased levels of reactive oxygen species/reactive nitrogen species. The result of these pathophysiological mechanisms is cell death (via apoptosis or necrosis), which the suppression of endogenous recovery mechanisms and promotes further axon degeneration and microcavity formation. c) The intermediate to chronic phase of spinal cord injury, which encompasses all time past the 2-week point, is characterized by the establishment of an “equilibrium state” where the injury environment subsides after significant white/gray matter degeneration and syrinx formation. In these stages, Wallerian degeneration, activation of microglia, and persistent pro-inflammatory cytokines play a key role in the pathophysiology and continue to inhibit the recovery response. d) Overview of the systemic complications that result from the degenerated spinal cord following injury, including respiratory, cardiovascular, gastrointestinal, reproductive, urinary, and musculoskeletal system issues. Figure taken from (Non-invasive approaches to functional recovery after spinal cord injury: Therapeutic targets and multimodal device interventions).[12]

Methylprednisolone/NASCIS Trials

In the late 20th century, the therapeutic potential of high-dose glucocorticoid treatment of spinal cord injury was evaluated by a number of experimental studies.[12,34] It was demonstrated through animal models of SCI that the glucocorticoid methylprednisolone (MP) could minimize SI by mitigating oxidative damage/ischemia and improving spinal cord blood flow.[12,33,34] These experimental results were then translated to a randomized controlled trial of 330 patients with acute traumatic SCI, called "The National Acute Spinal Cord Injury Study" (NASCIS I).[35] This multicenter double-blind randomized trial evaluated the effects of high-dose methylprednisolone treatment compared to a standard dose over a 10 day period post-SCI.[35] While there were no statistically significant results between the two groups of treatment, the clinical anti-inflammatory and antiedemic effects of MP facilitated an additional trial comparing MP, naloxone, and placebo treatment in NASCIS II.[36] The second trial revealed significant benefits in motor function, sensation to pinprick and touch compared to controls six months after high-dose MP treatment (30 mg/kg i.v. bolus, plus a 23-h infusion at 5 mg/kg/h).[36] Reported adverse side effects in NASCIS II following MP treatment were consistent with steroid therapy (i.e., gastrointestinal bleeding, wound infections, and delayed wound healing) but were not reported in the study at a statistically higher frequency compared to controls.[12,36] The use of MP as the standard for acute SCI care following the NASCIS trials quickly became utilized in clinical settings worldwide. Study limitations involving placebo groups (not used in NASCIS III due to ethical concerns of withholding treatment for patients with SCI that had reported advantages) prevented registered drug approval status from the Food and Drug Administration.[12,37] In 2013, after further studies linked MP treatment with higher rates of severe complications, the American Association of Neurological Surgeons and the Congress of Neurological Surgeons made the joint recommendation to no longer use methylprednisolone as the standard for acute SCI management.[33] As a result, there remains a need to identify effective therapeutic treatments to improve functional recovery in patients who sustain SCI.

Clinical Treatment of SCI: Overview

The current management of SCI consists of integrated trauma networks that link emergency medical services (EMS) and clinical providers to coordinate pre-hospital and intensive care unit treatment. EMS personnel are trained to rapidly assess and triage any potential traumatic brain or SCI while transporting the patient to an appropriate care facility. Once the patient has been admitted, efficient evaluation and stabilization methods are performed by clinicians, followed by operative decision-making (Table 1).[38] Surgical methods (e.g., decompression laminectomy, removal of bone fragments and foreign objects, and/or placement of spinal braces) are used once the patient is in a stable condition in order to remove excess fluid, tissue, or bone.[39,40] Following the acute period, the patient will typically undergo extensive rehabilitation and physical therapy to help promote as much functional independence through the use of assistive devices, muscle strengthening, self-grooming, and bladder and bowel management.[11]

While the prognosis for patients who experience traumatic SCI has improved in the acute setting, there remains a pressing need to devise therapies that can mitigate damage and effectively restore function of the somatic and autonomic nervous systems.[21] The challenges to developing highly effective therapies, however, are due to five major barriers to repair: 1) lengthy secondary injury processes, 2) inhibitory environment for neural recovery activities, 3) insufficient endogenous trophic and antioxidant support, 4) inadequate regeneration of adult CNS cells, and 5) lack of spontaneous activation of compensatory neural circuits to reactivate locomotor and other motosensory functions. [12,31,21] Many experimental and clinical trials are currently underway (highlighted in a review article by Badhiwala et. al. 2018), many of which focus on targeting secondary injury mechanisms to limit SCI lesion expansion and promote a more favorable endogenous recovery environment.[41] The current research on SI mechanisms highlights their therapeutic potential and each specific mechanism will be systematically evaluated to determine possible areas for intervention.

Table 1. Key Steps in the Management of a Patient with Acute Traumatic SCI.

Table taken from (Current Topics in the Management of Acute Traumatic Spinal Cord Injury).[38] *ABC airway, breathing, circulation; CT computed tomography; EMS emergency medical services; MAP mean arterial pressure; MRI magnetic resonance imaging; SCI spinal cord injury*.

1. Resuscitate (protect airway, maintain normotension), immobilize at scene (EMS)
2. Rapid transport to nearest *definitive* SCI care facility
3. ABC's, MAP management (85 mmHg)
4. No steroids
5. Radiographic assessment (CT initial study of choice)
6. Promptly realign cervical spine (closed vs open reduction)
7. Immobilize (external vs internal orthosis)
8. Maintain MAP perfusion parameters
9. MRI for mass lesion, extent of cord injury
10. Operative decompression early. Delay only for stabilization

Specific Aims

The specific aims of the following thesis include:

1. Highlight the normal physiology of the spinal cord and compare it to the different categories of secondary pathophysiological events (vascular system, immune system, neuronal system, and cellular systems) associated with traumatic injury with discussion of their key features.
2. Provide an overview of the physiology of oxidative molecules and their sub-cellular and cellular producers. Then, identify oxidative damage as a key role player in the progression of SCI by acting as 1) triggers, 2) mediators, and 3) effectors of neuroinflammation.
3. Discuss current research on representative ROS and RNS: Hydrogen Peroxide and Peroxynitrite. Identify the therapeutic studies targeting these key representative molecules. Then, discuss the mechanisms in which they may attenuate secondary spinal cord injury damage and promote endogenous recovery.

SECONDARY INJURY PROCESSES: REVIEW

Vascular System

This section will first review the normal physiology of the spinal cord's blood supply, including the major arteries, vessels, and the blood-spinal cord barrier. This section will then explore the ways in which the normal vasculature becomes disrupted following spinal cord injury, leading to multiple secondary injury mechanisms including hemorrhagic necrosis, ischemia, and edema (Table 2).

Physiology of Spinal Cord Vasculature

The vasculature that supplies the spinal cord is composed of a complex array of arteries, veins, and microvasculature. The major arteries supply blood to a large density of microvessels in the deep neural parenchyma of the gray matter, providing nourishment and removing waste to the neuronal and glial cells.[42] The blood-spinal cord barrier (BSCB) is an important protective feature of the spinal cord that functions by tightly regulating spinal cord homeostasis, serving a protective and regulatory role for the spinal cord parenchyma.[43] The structural components of the BSCB that provide the cord with a high degree of selective permeability are non-fenestrated endothelial cells, pericytes, and astrocytes. Disruption of this highly regulated and sequestered vascular system cause further damage to the cord via SI processes (hemorrhagic necrosis, ischemia, and vasogenic or cytotoxic edema).[42]

Hemorrhagic Necrosis

Hemorrhagic necrosis is one of the earliest occurring secondary events following the primary mechanical insult to the cord. Numerous clinical and experimental research studies of SCI have identified rapid hemorrhage progression during the acute (0-48 hours) phase of injury.[24,44,45,46] Mechanical damage to blood vessels following the traumatic insult initiates hemorrhage (i.e., release of erythrocytes and inflammatory cells).[47] Introduction of these vascular components into the spinal cord parenchyma causes chemical and physical stress, resulting in necrotic cell death.[47,48] Histological tissues obtained from human cadavers following traumatic SCI revealed marked intramedullary hemorrhages mainly in the gray matter, with pathologic presence of extravasated red blood cells and polymorphonuclear cells.[42] Much of this hemorrhage, as discussed by Tator and Koyanagi, is due to extensive occlusion and vasospasm that raises the pressure inside the intramedullary vessels and causes them to rupture.[42] Studies utilizing more advanced imaging techniques (MRI/CT angiography and synchrotron radiation micro-computed tomography [SRμCT]) have been able to further validate the presence and patterns of SCI hemorrhage in gray matter.[44,49] Utilization of SRμCT, a high resolution 3D imaging technique, demonstrated both the progressive and central nature of hemorrhage as it spreads in the rostral and caudal direction from the lesion center following injury.[47,49] The leakage of erythrocytes introduces heme degradation products, namely iron, to the gray matter of the cord. The high reactivity of iron instigates

SI oxidative damage, generating reactive oxygen species that contribute to major cellular disruptions and necrosis following hemorrhage.[48,50] In addition to the introduction of deleterious agents, hemorrhage also results in vasogenic edema from fluid accumulation in the extracellular space, hypoperfusion-induced ischemia from disrupted blood flow, and immune system activation following necrotic-release of damage-associated molecular patterns (DAMPs) that are recognized by immune cells and trigger an inflammatory response.[42,46]

Ischemia

Posttraumatic spinal cord ischemia is another vascular SI event that occurs acutely after SCI. Ischemia is marked by the inadequate blood supply to tissues, resulting in reduced metabolic production of ATP, increased glycolysis and intracellular acidity (from glycolytic production of pyruvic acid), and cell death.[51] Multiple compensatory and destructive vascular mechanisms that act following injury such as hemorrhage, thrombosis, vasospasm, and disruption of the microcirculation (characterized by increased endothelial permeability), are believed to contribute to the presence of ischemia in the spinal cord.[22,52,53] Dr. Justin A. Zivin, a neurologist and researcher based in San Diego, California developed a rabbit model of spinal cord ischemia by performing an occlusion of the aorta for various time intervals, which resulted in varying degrees of paraplegia.[54] This study, along with many other ones using methods of angiography, blood flow techniques, and transmission/scanning electron microscopy have provided further demonstrated an ischemic vascular response following spinal cord injury.[22,42,54,55,56] SCI-related ischemia is a confluence of multiple vascular mechanisms, but the resultant reductions in blood flow, perfusion, and oxygenation leads to widespread tissue death and axonal dysfunction following SCI.[3,57]

Edema

The presence of edema in acute SCI originates from multiple different pathophysiological sources following the disruption of the BSCB.[58] Edema can be characterized as either vasogenic (extracellular accumulation of fluid) or cytotoxic (intracellular accumulation of fluid) and both types are present in SCI.[58] Cytotoxic edema in SCI has been shown to ionic disturbances involving rapid intracellular uptake of Na^+ and water.[58,59] Vasogenic edema in SCI results from mechanical disruption of the BSCB and other vasculature, and has been observed in experimental models of SCI utilizing histological and fluorescent tracing methods.[60,61] A separate study by Yashon et. al. characterized parenchymal tissue edema in rhesus monkey models of SCI. Edema was measured using a ratio of water content to nonvolatile residue (material remaining following evaporation of the volatile solvent which contains the material) per unit weight, which was shown to rapidly increase within 6 hours post-traumatic insult to the spinal cord.[62] Presence of edematous plasma exudate (consisting of erythrocytes, proteins, and leukocytes) in the parenchymal tissues can increase mechanical compression of dura mater and elevate intraspinal pressure, which contributes to further tissue degeneration by promoting ischemia and metabolic-related cell death pathways.[58,61]

Immune/Inflammation System

This section will first review the normal physiology of the immune response in the spinal cord, including descriptions of the endogenous and peripheral immune cells and the normal course of neuroinflammation (mediated by resident immune cells) and inflammation (mediated by peripheral immune cells). This section will then explore the ways in which the immune system becomes dysregulated following SCI, resulting in multiple SI mechanisms including cytokine release, residential neuroinflammatory response by microglia and astrocytes, and peripheral inflammatory response by neutrophils, macrophages and lymphocytes (Table 2).

Physiology of Spinal Cord Immune Response

The immune system regulation of the spinal cord consists of endogenous immune cells and circulating immune cells that function to control infection, clear cellular debris, promote wound healing, and regulate systemic homeostasis as part of neuroinflammation.[63] The endogenous (resident) immune cells (i.e., microglia and astrocytes) which act as immune surveillance cells in the CNS parenchyma. These cells serve as a “first line of defense” for any foreign pathogens that infiltrate the cord.[64] The peripherally circulating immune cells (i.e., neutrophils, macrophages, and lymphocytes) in contrast, are located outside the BSCB in the blood stream.[64] The inflammatory response is activated when specific signaling molecules, called proinflammatory cytokines and chemokines, are released by neurons, oligodendrocytes, microglia, astrocytes, and peripheral macrophages in response to damage associated molecular patterns (DAMPs) from SCI. The released signaling molecules then recruit additional immune cells to respond to the foreign pathogen, toxic substance, or injury that caused the release of the DAMPs.[65] Typically, the immune cells respond and carry out their functions in an appropriate fashion and then are “turned off” by anti-inflammatory and pro-resolution molecules that establish a pro-recovery environment before restoring homeostasis.[65] In SCI, there is an imbalance favoring pro-inflammatory markers due to the continuous damage from other unregulated SI processes. The overactive inflammatory response in SCI is thus characterized by elevated levels of cytokines and immune cells, subsequent edema, activation of astrocytes and microglia, and eventual glial scar formation.[63,66,67,68,69,70]

Cytokine Release

Proinflammatory cytokine release, otherwise known as the cytokine cascade, is another acute secondary injury mechanism of spinal cord injury.[63] Cytokines are small proteins that function as signaling molecules for immune cells (i.e., Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6) and become significantly upregulated immediately after injury.[71,72] Along with their role in immune cell recruitment, these cytokines promote SI damage by exacerbating the lesion size through activation of apoptosis and necrosis, enhancing excitotoxicity, and increasing edema. Multiple experimental studies of animals and clinical investigations have identified upregulated levels of proinflammatory cytokines in the extracellular space following SCI.[63,72,73,74] A study by Pineau and Lacroix found that experimental SCI in mice lead to a rapid and transient expression of proinflammatory cytokines using *in situ* hybridization to detect mRNA.[72] Cytokine mRNA was shown to be upregulated as early as 5 minutes (IL-1β), 15 minutes (TNF-α), and 3 hours (IL-6) post-SCI.[72] The role of endogenous CNS cells on cytokine expression were described by Yang et. al 2004. in a histological study of human spinal cord tissue, which showed that there was increased immunoreactivity of IL-1β, IL-6, and TNF-α in neurons 30 minutes after injury.[74] In a study of a rat model of SCI conducted by Yang et. al. 2005, levels and temporal development of TNF-α, IL-1β, and IL-6 were verified by histological analysis, reverse transcription polymerase chain reaction (RT-PCR), and western blot analysis.[73] Their study described the role of endogenous CNS cells (neurons, astrocytes, microglia, and oligodendrocytes) as initiators of inflammation by releasing these cytokines to recruit peripheral immune cells.[72,73] Following the initial release of cytokines, there is a further "cytokine cascade" that occurs as a result of cytokine-releasing inflammatory cells that infiltrate the site of injury, leading to subsequent deleterious events such as persistent edema, initiation of cell death pathways, and increased production of reactive oxygen/nitrogen species.[63,68,72,73]

Residential Inflammatory Response

Residential inflammatory cells are some of the first cells to actively respond following spinal cord trauma (Figure 2). DAMPs are immediately released by injured cells (e.g., neurons, oligodendrocytes, microglia, astrocytes) following the traumatic insult to the spinal cord, signaling normally quiescent microglia cells to become activated.[25,75] The activated microglia undergo hypertrophic morphological and functional changes to polarize into the M1 (proinflammatory), M2 (anti-inflammatory), and other intermediate phenotypes. Presence of either and other polarization phenotypes can be observed following SCI, as the M1/M2 macrophages are believed to exist on a dynamic continuum.[25,76] A study by Fleming et. al. utilized immunohistochemical methods to evaluate levels of the cellular inflammatory response in post-mortem human spinal cord tissue post-injury. This study identified the elevated presence of activated microglia at 1-3 days post-injury, localized mostly in the gray matter of the spinal cord.[77] Studies in rat models of spinal cord injury have also demonstrated the presence and time course of activated microglia, using methods of flow cytometry and immunohistochemistry.[67,78,79] Microglial activation has multiple modes of action, as it can 1) initiate a cascade of neurotoxic responses through upregulation of ROS and proinflammatory cytokines, 2) induce other microglia and astrocytes to undergo their own activation, or 3) release anti-inflammatory markers to support resolution of the immune response.[72,80,81]

Astrocytes play an important role in the BSCB and help to maintain homeostasis of the CNS by regulating levels of neurotransmitters and communication with neuronal cells and between neurons through its processes in the perisynaptic space.[82] When they become activated, they display similar phenotypic changes and function to microglia, releasing a cascade of proinflammatory cytokines and reactive oxygen species.[72,80] One of the most important functions of reactive astrocytes is their proliferation, migration and formation of a reactive gliosis zone (also called the glial scar).[69,81,83,84,85] The migration of astrocytes has been demonstrated in mice models of SCI, where immunohistochemical analysis of the glial fibrillary acidic protein (GFAP) that is expressed by astrocytes was found to be elevated and on the vicinity of the lesion.[69,80,84,86] The enclosure of the lesion by astrocytes is an important component of recovery, providing a dense astrocytic barrier that isolates the site of primary injury and limits the damaging effects of exacerbated peripheral immune response.[66] This barrier, debatably, may also play a role in beneficial neuroplasticity and perhaps axonal regeneration (with the latter being a controversial topic).[66,69,84] Neuroinflammation, composed of the microglia and astrocytes injury response, are part of the overwhelming cascade of inflammatory response and the enclosure of the lesion, and perform dual roles in exacerbating or resolving injury.

Peripheral Inflammatory Cell Response

Peripheral inflammatory cells (i.e., neutrophils, macrophages, and lymphocytes) first enter the site of traumatic SCI immediately following the injury as vascular disruptions leak previously circulating immune cells into the lesion zone. The main influx of inflammatory cells, however, arrives shortly after the release of cytokines by resident CNS cells.[25,63,68,72] The infiltration of inflammatory cells from blood vessels occurs through a regulated process of leukocyte extravasation. Selectin molecules on the endothelial wall of the blood vessels facilitate “rolling” of circulating immune cells to slow them down, followed by adhesion to intercellular adhesion molecules (ICAM) that helps promote transendothelial migration into the lesion site. Each type of immune cells (both peripheral and residential) has a different temporal sequence as part of the inflammatory response to SCI (Figure 2).[25,63,77,78,87,88,89] An observation of these different time courses was made in a study by Fleming et. al. (2006) using highly selective immunohistochemical markers. Neutrophil infiltration was initiated within the first 3-4 hours and peaked between 1-3 days post-injury.[77] Macrophages were activated and entered the lesion site within the first 1-3 days, elevated to higher levels 5-10 days post-injury, and remained elevated for up to 6 months after.[77] Lymphocytes were only seen at elevated levels 1-3 months following injury and are mostly present at the vicinity of the lesion.[77] These results are consistent with two additionally evaluated experimental studies of inflammation that used methods of flow cytometry, immunohistochemistry, MRI imaging and other techniques to identify the time course of inflammatory cells in the SCI environment.[78,90] Neutrophils and macrophages play a significant role in the course of SI, as their elevated levels upregulate oxidative and proteasomes via enzymatic production, resulting in bystander tissue damage (non-specific destruction of tissue by immune cells).[87,88,89] Both cell types also play a role in recovery of the spinal cord, including clearing of tissue debris and other anti-inflammatory roles.[91,92] In acute traumatic SCI, however, the overwhelming presence of inflammatory cells, coupled with other SI mechanisms, increases neurotoxicity.[89]

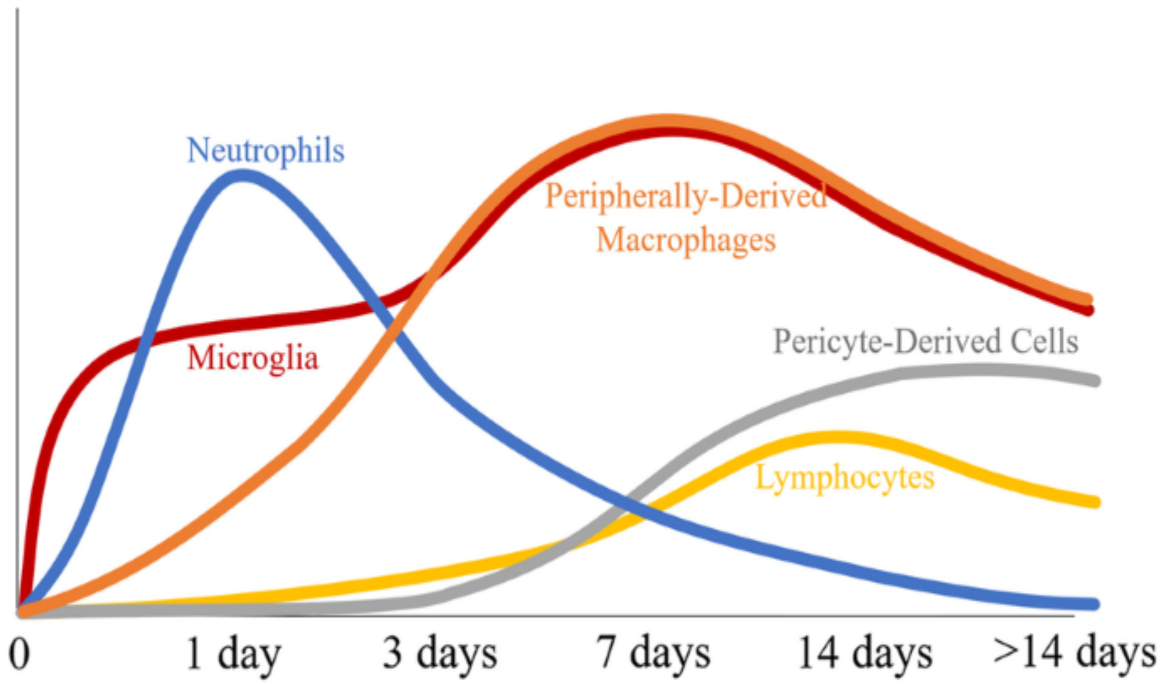

Figure 2. Time Course of Inflammatory Response Post SCI.
Depiction of the varying responses to SCI by each immune cell. Resident CNS microglia are the earliest responders to the primary insult. Peripheral macrophages infiltrate mostly after day one of injury and are morphologically identical to resident microglia. The combination of resident microglia/peripheral macrophages maintain elevated levels for >14 days following injury. Neutrophils are quickly directed to the lesion and peak around day 1 post-injury and decrease gradually until about day 10. Lymphocytes and pericyte-derived cells peak at lower levels and much later than the other immune cells. Figure taken from (Inflammation after spinal cord injury: a review of the critical timeline of signaling cues and cellular infiltration).[67]

Lesion Expansion: Neuroinflammation as Key Role Player

Neuroinflammation in the spinal cord plays a double-edged role following traumatic injury, either by engaging in tissue repair activities or contributing to the continued destruction of tissue.[93] The recovery or destructive driven nature of inflammation is dictated by the context, duration, and influence of other pathophysiological injury mechanisms of SCI.[93] Inflammation can exert a beneficial impact following SCI by mediating the clearance of cellular debris through phagocytic mechanisms.[94] Specific inflammatory cells, including microglia and macrophages, also have the ability to undergo phenotypic changes that can mediate an anti-inflammatory/pro-resolution response following injury.[65,76] Despite these beneficial effects, inflammation in SCI appears to mostly exert detrimental effects, as inflammatory molecules prolong the tissue damage in the lesion core and surrounding lesion rim in the acute-chronic phases of injury.[93,94,95] The widespread spatiotemporal presence of inflammatory cells in SCI autopsy tissues, demonstrated in a study by Zrzavy et. al. in provides evidence of their fundamental role in tissue damage post-injury.[94] Their study additionally highlighted evidence of oxidative tissue injury using immunohistochemical analysis, demonstrating that markers of oxidative damage were found in the rim surrounding the necrotic core of the lesion in a pattern similar to inflammatory cell presence.[94] The presence of oxidative damage studied by Zrzavy, characterized by using oxidized lipids as established markers for neuronal cell body and axonal injury, persisted in the lesion core for months following SCI and for over a year in the lesion rim, indicating an important oxidative-neuroinflammatory relationship that contributes to impaired recovery and may serve as a target for therapeutic interventions.[94]

Neuronal System

This section will first review the normal physiology of the spinal cord's neural signal relay, including the basic neuroanatomy of the cord and the basic physiology of neurotransmission (i.e., the transmission of nerve impulses between neurons or between a neuron and muscle fiber). This section will then explore the ways in which the intrinsic functions of the cellular components in the nervous system contribute to neurodegeneration following spinal cord injury, via multiple SI mechanisms including excitotoxicity and axon/neurite degeneration (Table 2).

Physiology of Spinal Cord Signal Transmission

The main function of the spinal cord is to act as a neural signal transduction conduit between the peripheral nervous system (PNS) and the CNS (i.e., the brain and spinal cord).[96] The spinal cord processes, integrates, modulates, and relays three different classes of neural signals: motor (e.g., voluntary and other muscle movements), sensory (e.g., somatic sensation of touch, pressure, temperature, pain, vibration, and proprioception; visceral sensation; etc.), and autonomic afferent and efferent signals (e.g., concerning digestion, urination, body temperature, heart rate, blood pressure, etc.).[96] At the cellular level, communication between neurons occurs through a process involving chemical transduction across a cell synapse and electrical transduction through axons.[97] One neuron will receive chemical signals through its dendrites, that represents either an excitatory or inhibitory signal for the receiving neuron.[97] When a certain excitatory threshold at the receiving neuron is met, an action potential will travel along the axon by mainly opening and closing of specific Na^+, K^+, Ca^{2+}, and Cl^- channels. Once the action potential reaches the presynaptic membrane of the synaptic cleft, the resultant depolarization signals Ca^{2+} gated channels to open, increasing the intracellular Ca^{2+} concentration and releasing neurotransmitters (e.g., glutamate for excitatory signaling or glycine for inhibitory signaling) to interact with the specific receptors on the postsynaptic membrane.[97] This simplified version of the process nevertheless highlights the nature of neurotransmission that is done via stringently regulated electrical and chemical activities to propagate neural signals throughout the body. This system of signal generation and the network of neurocircuits that rely on functional neurotransmission, is severely altered in the course of SCI, and leading to conduction block and sensorimotor deficits.[96]

Excitotoxicity

During the acute phase of SCI, extracellular levels of neurotransmitters, specifically glutamate, are highly elevated by the disruption of numerous glutamate sources .[26] Glutamate is the major excitatory neurotransmitter in the spinal cord and in acute SCI can arise from necrotic neurons, axon cylinders, and astrocytes.[26] Glutamate acts on a variety of ionotropic receptors that are characterized into three classes: α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA), kainite (KA), and N-methyl-D-aspartate (NMDA) receptors.[26,98] NMDA receptors are found primarily on neurons, whereas AMPA-kainate receptors can be found on neurons, oligodendrocytes, and astrocytes.[99,100] All of these ionotropic receptors are activated by glutamate and, when activated, regulate the transport of Ca^{2+} and Na^{+} ions into the post-synaptic cell.[100] Glutamate's role in neurotoxicity following SCI has been extensively studied, with *in vitro* and *in vivo* evidence supporting glutamate-mediated initiation of necrosis and apoptosis by increasing levels of ROS and RNS in the post-synaptic neurons during the acute phase of injury.[26,99,101,102,103] Overactivation of glutamate release causes a rapid influx of Ca^{2+} and Na^{+} into the cells, resulting in subsequent cytotoxic edema, intracellular acidosis, activation of proteinases, and apoptosis and/or necrosis pathway activation[22] The damage caused by the rapid influx of Ca^{2+} into gray and white matter tissue affects the neurons, oligodendrocytes, and astrocytes that express these the NMDA, AMPA, and KA receptors.[26,99,103] Furthermore, it has been shown in studies of SCI that a secondary pathway of excitotoxic damage is through the generation of reactive oxygen species following elevated levels of cytosolic Ca^{2+}.[104] Finally, the dysregulation of glutamate and ion channels leads to conduction block, which prevents neuronal signals from traversing the site of the lesion, even if some axonal projections remain intact.[105]

Axon Degeneration

Axon degeneration is a secondary injury event that consists of the progressive degeneration of nerves in the subacute (2-14 days post-injury) phase of SCI.[106] Experiments conducted in 1851 by Dr. Augustus Waller, a British neurophysiologist, were the first to identify the occurrence of nerve degeneration following transection of the hypoglossal and glossopharyngeal nerves in adult frogs.[106] Axon degeneration is the key characteristic feature of wallerian degeneration, but has characteristic differences in the PNS and CNS which contribute to the detrimental pathophysiology of SCI.[107,108] In the PNS, Wallerian degeneration resolves much faster due to the permeability of the blood-tissue barrier. This relatively higher permeability allows Schwann cells and macrophages to infiltrate and scavenge the myelin debris. In the CNS, microglia and oligodendrocytes have been found to be the cells involved in attempting wallerian degeneration, as peripheral cells are not able to infiltrate the BSCB. The ability of Schwann cells to break down the myelin sheath and remove debris plays a fundamental role in PNS Wallerian degeneration.[109] Oligodendrocytes, in contrast, have very little documented ability to clear myelin or axon debris, which is a critical step to drive regeneration of the nerve after injury. This concept was demonstrated in a study by Ludwin in an optic nerve injury in mice. His study showed, via immunocytochemical methods, that oligodendrocytes are present at the site of the injury, but there is little to no evidence of remyelination or oligodendrocyte-driven phagocytosis.[110] In a radiation bone marrow chimeric rat study by Popovich and Hickey, microglia were shown to be present at the regions of wallerian degeneration in a contused spinal cord model. These microglia, however, are not seemingly activated, evidenced by the downregulated expression of activated myelin phagocytosing macrophages markers.[111] Reichert and Rotshenker provide further evidence of this point in an *in vivo* optic nerve injury in rats, which showed that expression of MAC-2 (a macrophage/monocyte activation marker) was not found in microglial cells surrounding the injured optic nerve.[112] The reduced ability of the CNS to clear debris and regenerate axons following axon degeneration, based on the intrinsic properties of oligodendrocytes and microglia, leads to poor regeneration of axons and contributes to the functional deficits in SCI.[66]

Cellular Systems

This section will first review the normal physiology of cell homeostasis in the spinal cord, highlighting the crucial role of mitochondria and ion regulation for regulating neuronal signal transduction. This section will then explore the ways in which the mitochondrial and ionic homeostatic systems become dysregulated, leading to multiple secondary injury mechanisms including mitochondrial dysfunction, ionic disturbances, necrosis and apoptosis, and oxidative damage (Table 2).

Cell Physiology

Every cell has a tightly regulated set of processes that dictate energy production, cell-to-cell communication, intracellular and extracellular transport, and maintenance of ion balance. All these regulatory mechanisms function through well-studied cellular biology pathways and subcellular biochemical reactions involving the nucleus, mitochondria, and ion channels. In the nervous system, mitochondria and voltage-gated channels play an especially important role given the large energy demands and ion channel function required for signal transduction.[113,114] The mitochondria is an intracellular organelle that converts ADP to ATP through the process of oxidative phosphorylation, providing the cell with vital stores of energy to carry out different functions. Many ion channels (such as Na^+/K^+-ATPase pump and Ca^{2+} transport ATPases) rely on ATP to drive the flow of ions against their concentration gradients and are thus reliant on the mitochondria to maintain homeostasis.[115,116] When the mitochondria and ion channels become dysregulated, there are cellular mechanisms in place to either attempt repair or sacrifice the cells (i.e., apoptotic or necrotic cell death) to prevent any worsening damage.[29] In SCI, normal mitochondrial and ionic mechanisms are impacted and lead to the progression of secondary injury mechanisms resulting in chronic detrimental changes to the spinal cord microenvironment.

Mitochondrial Dysfunction

Mitochondrial dysfunction is an important secondary mechanism that occurs during the acute-subacute phase of SCI.[117] The mitochondria in the CNS play an important role in generating ATP, but are also critical for Ca^{2+} regulation, cell apoptosis signaling, and production/sequestration of reactive oxygen species.[118] During the sequelae of acute SCI, many of the secondary injury mechanisms have a detrimental impact on the normal functioning of the mitochondria, including glutamate-mediated excitotoxicity and ischemia.[51,104] The temporal changes in mitochondrial bioenergetics were evaluated in a study by Sullivan et. al. of spinal cord injuries in rats. By measuring levels of 3-nitrotyrosine or 4-hydroxynonenal as mitochondrial markers of oxidative damage and using a Clark-type oxygen electrode to measure mitochondrial respiration, the study demonstrated a progressive loss of mitochondrial function up to 24 hours post-injury.[117] The loss of mitochondrial respiration corresponds with the presence of ischemia in the cord, which interrupts the oxygen supply for aerobic respiration and results in a shift anaerobic metabolism.[51] Decreased ATP production then leads to dysregulation of Ca^{2+} -gated channels result in elevated levels of mitochondrial Ca^{2+} and upregulation of ROS and RNS molecules.[51] The coupled elevation of Ca^{2+} and oxidative molecules trigger the opening of the mitochondrial permeability transition pore (mPTP).[119] The opening of the mPTP then leads to a drastic decrease in the inner mitochondrial membrane potential and cessation of ATP synthesis.[119,120] The mPTP opening also increases the permeability of the mitochondrial double membrane, allowing molecules and water from the cytosol to enter and swell inside the mitochondria. When the membrane bursts, the mitochondria releases its contents into the cell, which includes accumulated Ca^{2+}, high generated levels of reactive oxygen species, and proapoptotic proteins (cytochrome *c*). [27,120,121] The release of cytochrome *c*, whether by mitochondrial lysis or detection of cellular damage, will then initiate the apoptotic cell death pathway.[27]

Ionic Disturbances

During the acute phase of SCI, the homeostasis of many important ions become dysregulated due to mitochondrial dysfunction and mechanical disruption of the cellular membrane.[122] The disturbance of these key ions is clearly highlighted in a review article by Liu et. al., which includes information on the specific ion channels and how they are altered post-injury.[123] The regulation of Na^+, K^+, Ca^{2+}, and Mg^{2+}, depends on the functionality of key ion transport channels, many of which are ATP-driven or rely on the ion concentration gradients that are established by ATP-driven pumps.[124] In SCI, concentrations of Na^+ and Ca^{2+} become upregulated intracellularly, while K^+ and Mg^{2+} become upregulated extracellularly.[28] Buildup of Na^+ inside the cell leads to a hypertonic solution that creates a gradient for water to flow into the cell, causing cytotoxic cellular edema.[59,125] This point was explored in a study by Lemke et. al. (1990) of an experimental spinal cord trauma model in rats. Tissue levels of the cations Na^+ and Mg^{2+} were measured using a spectrophotometer, air-acetylene flame, and hollow cathode Na^+/K^+ or Ca^{2+}/Mg^{2+} lamps, which revealed a correlation between elevated Na^+ and elevated water content following injury.[125] Elevated intracellular Na^+ has also been linked with reversal of the Na^+-H^+ exchanger and increased intracellular acidosis.[124] The role of K^+ in the pathophysiology of acute SCI results from disruption of myelin following injury. A study by Chiu and Richie found that axonal K^+ channels were concealed by the myelin sheath in the internodal membrane in a voltage-clamp experiment of rabbits.[126] They demonstrated that acute myelin loss leads to rapid release of intracellular K^+, which resets the axon's resting membrane potential (usually -70 mV) to the equilibrium potential of K^+ (-90 mV), which prevents action potentials from being generated and leads to conduction block.[127] Mg^{2+} plays a functional role as a physiological Ca^{2+} antagonist, maintaining high levels intracellularly to block NMDA receptors and help regulate Ca^{2+} levels.[125,128] Depletion of intracellular Mg^{2+} therefore leads to an exacerbation of excitotoxicity mediated by an uncontrolled rise in intracellular Ca^{2+}.[128] In a study of measured serum ionized magnesium concentrations in 30 patients with traumatic brain injury, conducted by Kahraman et. al., it was found that the magnitude of intracellular decline of Mg^{2+} are related to the severity of injury.[129] Ca^{2+} signals the release of cytochrome *c* and increased production of ROS and RNS molecules, initiating the apoptotic cell death cascade.[27] Treatment of primary cultures of adult rat hepatocytes with extracellular Ca^{2+} in a study by Schanne et. al., found that Ca^{2+} initiates a final common pathway for the toxic death of these hepatocytes. This study also demonstrated that there are two key steps involving calcium-mediated death, the disruption of the plasma membrane followed by the initiation of cell death pathways, which plays a fundamental role in neurotoxic lesion expansion in SCI.[130]

Necrosis and Apoptosis

The cell death cascades, necrosis and apoptosis, occur during the acute-subacute phase of SCI.[131] Apoptosis is a regulated biological process that involves an energy-dependent cascade of molecular events.[132] There are two main pathways that can lead to apoptosis, an extrinsic pathway (death receptor leads to activation of caspase 8/caspase 3) and an intrinsic pathway (mitochondrial changes leading to caspase 9/caspase 3 activation).[133] The notable morphological features of apoptosis includes cell shrinkage, intact cell membrane, apoptotic bodies that retain cytoplasm, and absence of subsequent inflammatory response.[133] In contrast, necrotic cell death involves cellular swelling, disruption of organelles, and membrane lysis that releases intracellular contents and initiates an inflammatory response.[131] Numerous studies, including a human postmortem study by Emery et. al., have shown presence of apoptosis and necrosis following SCI.[132,134,135,136] A study by Yong et. al. of a severe contusion injury in adult rat spinal cords found evidence of apoptosis in both gray and white matter of the cord using TUNEL staining and electron microscopy techniques. Their study revealed that neurons, astrocytes, and microglia all underwent apoptosis following SCI and reached a peak on day 3 after injury around the lesion site.[134] A similar study by Kato et. al. aimed to identify the role of neuronal death following spinal cord ischemia in a rat injury model. Using TUNEL staining and electron microscopy, they found that neuronal necrosis was present 6 hours post-injury and remained for as long as 7 days. They also found that neuronal apoptosis was present 1-2 days post-injury and remained for 4 days.[136] These two cell death models exist in a continuum based on magnitude and duration of the primary insult and resultant secondary injury mechanisms. Necrosis is found when cell stress signals overwhelm the cells, or if there is a lack of ATP generation, whereas apoptosis occurs when there is ATP and carefully controlled signaling.[29,131] Both pathways can be initiated following ischemia, glutamate excitotoxicity, reactive oxygen species, and other secondary injury mechanisms and are markers of the continued lesion expansion in SCI.[29]

Oxidative Damage

Oxidative damage occurs in the acute-subacute phase of SCI and is one of the main mediators of damage to neuronal cells.[137] ROS and RNS are either free radical (containing an unpaired electron) or highly reactive species (containing chemically reactive electrons) that undergo oxidation-reduction reactions through the exchange of electrons with surrounding proteins, enzymes, receptors, channels, membranes, or nucleic acids.[138] ROS and RNS originate from multiple secondary injury sources following SCI, including mitochondrial dysfunction, glutamate excitotoxicity, loss of ionic homeostasis, microvascular disruption, and inflammation.[138,139,140] The main identified oxidative species involved in SCI are Ferric/Ferrous forms of iron (Fe^{2+}/Fe^{3+}), Nitric oxide ($\cdot NO$), Hydroxyl radical ($OH\cdot$), Peroxynitrite ($ONOO^-$), Superoxide ($O_2\cdot^-$), Hydrogen peroxide (H_2O_2), Hypochlorous acid (HOCl), and Lipid Radicals ($LOO\cdot$/$LO\cdot$).[30,141,142,143] These species react with proteins, lipids, and DNA, which results in the initiation of the lipid peroxidation cascade, loss of membrane fluidity, and eventual cell necrosis and apoptosis.[140] The production of these molecules, given their high levels of reactivity, can also undergo cascades of reactions with each other, that can lead to their prolonged presence in SCI. Under normal physiological circumstance, these species are tightly regulated through a series of oxidation/reduction reactions and serve many important functions that will be covered in later sections. In the context of SCI, however, the antioxidant mechanisms that control the levels of oxidative species becomes rapidly overwhelmed, leading to considerable damage in the spinal cord.[140]

Table 2. Overview of Acute and Subacute Secondary Injury Mechanisms

Summary of the acute/subacute secondary injury mechanisms that occur following spinal cord injury. *BSCB – Blood Spinal Cord Barrier, ROS – Reactive Oxygen Species, RNS – Reactive Nitrogen Species*.

2° Injury Pathophysiology	Biological Category	Time Course	Cellular/Molecular Events	Downstream Effects
Hemorrhagic Necrosis	Vascular System	Acute	• Endothelial damage[47,48] • Heme Degradation and Increased $[Fe]_e$[48,50]	• Edema[46] • Inflammatory Response[42,46] • Tissue Death[46]
Ischemia	Vascular System	Acute	• Reduced Perfusion[51] • Increased Endothelial Cell Permeability[22,53] • Thrombosis/Vasospasm[52,53]	• Decreased Metabolism[51] • Increased Intracellular Acidity[51] • Cell Death[51]
Edema	Vascular System	Acute	• Disruption of BSCB[58] • Infiltration of Erythrocytes, Leukocytes, and Proteins[58,61]	• Mechanical Compression[58,61] • Increased Intraspinal Pressure[58,61] • Ischemia[58,61]
Cytokine Release	Immune System	Acute	• ↑ Tumor Necrosis Factor (TNF)[72] • ↑ Interleukin-1β (IL-1β)[72] • ↑ Interleukin-6 (IL-6)[72]	• Inflammatory Cell Recruitment[63,68] • Increased ROS/RNS[63,68] • Cell Death[63,68]
Residential Inflammatory Cell Response	Immune System	Acute Subacute	• Activated Microglia[25,75] • Reactive Astrocytes[72,80]	• Increased ROS/RNS[72,80] • Glial Scar Formation[66,69,84] • Axon Regeneration Inhibition[66,69]
Peripheral Inflammatory Cell Response	Immune System	Acute Subacute	• Neutrophil Recruitment[25] • Monocyte/Macrophage Recruitment[25] • Lymphocyte Infiltration[25]	• Release of Oxidative and Proteolytic Enzymes[87,88,89] • Tissue Damage/Lesion Expansion[87,88,89] • Removal of Tissue Debris[91,92]

Table 2. Overview of Acute and Subacute Secondary Injury Mechanisms Continued

2° Injury Pathophysiology	Biological Category	Time Course	Cellular/Molecular Events	Downstream Effects
Excitotoxicity	Neuronal System	Acute	• Excessive Release of Glutamate[26] • Overactivation of NMDA/AMPA/KA Receptors[26,98]	• Rapid Influx of $[Na^+]_i$ and $[Ca^2]_i$[26,99,103] • Cytotoxic Edema[22] • Cell Death[22]
Axon Degeneration	Neuronal System	Subacute	• Fragmentation of Myelin[108] • Reduced Clearance of Myelin Debris[110]	• Poor Remyelination/Axon Regeneration[66,110] • Conduction Block[108]
Mitochondrial Dysfunction	Metabolic System	Acute	• Diminished ATP Production[119,120] • Increased Permeability of Mitochondrial Membrane[119,120]	• Release of Cytochrome *c*, ROS, and Ca^{2+} Intracellularly[27,120,121] • Cell Death[27]
Apoptosis/Necrosis	Cell Death	Acute Subacute	• ATP-dependent Cell Death Pathway (Apoptosis)[132,133] • "Suicide" Cell Death Pathway (Necrosis)[131]	• Tissue Death[132] • Release of Intracellular Contents[131] • Inflammatory Response[131]
Na^+/Ca^{2+} Influx	Ionic Disturbances	Acute	• Failure of Na^+/K^+ Pump[124] • Glutamate Excitotoxicity[27]	• Cytotoxic Edema[125] • Increased Intracellular Acidosis[124] • Cell Death[27]
K^+/Mg^{2+} Efflux	Ionic Disturbances	Acute	• Disruption of Myelin and K^+ Leak Channels[127] • Overactivation of NMDA/AMPA/KA Receptors[125,128]	• Conduction Block[127] • Increased Excitotoxicity[128]
Increased ROS/RNS	Oxidative Damage	Subacute	• Superoxide (O_2•)[141] • Nitric Oxide (NO•)[141] • Hydroxyl Radical (OH•)[141] • Peroxynitrite ($ONOO^-$)[129,141] • Hydrogen Peroxide (H_2O_2)[141]	• Loss of Membrane Fluidity[140] • Lipid Peroxidation Cascade[140] • Increased Neuroinflammation[94] • Cell Death[140]

OXIDATIVE CASCADES

Oxidative Molecules: Role in Physiology

ROS, for many years, were believed to be unregulated mediators of intracellular damage. While molecular oxygen attracted a positive view in the early 20th century because of its role in cellular respiration and generation of ATP, ROS were implicated as potential contributors to numerous pathologies by damaging DNA, proteins, and lipids through oxidation.[144,145,146] However, it has since been shown that ROS perform a dual role in physiology by acting as key cell signaling molecules at low, steady-state concentrations or as mediators of damage at higher concentrations, as highlighted in a review article by Lambeth in 2007.[147] As more evidence of the physiological role of reactive oxygen species has been uncovered, important sub-organelles responsible for producing them have been emphasized, including the mitochondria, peroxisomes, and endoplasmic reticulum (ER) which help to validate their contributions to functional biological processes.[148] In the last 10 years, these three sub-organelles have been shown in a number of studies to have significant redox “cross-talk” capabilities through inter-organelle membrane connections and cycling of specific oxidoreductase enzymes and protein chaperones that are able to sense levels of redox balance intracellularly.[149] The physiological role of RNS has undergone a similar frameshift in the 20th century, after Ferid Murad postulated that the mechanism of guanylate cyclase activation was due to the release of nitric oxide from nitroglycerin, which introduced research into nitric oxide as a chemical vasodilator.[150,151] The generation of NO is also mediated by nitric oxide synthase (NOS) enzymes which are believed to have four distinct isoforms: inducible (iNOS), endothelial (eNOS), neuronal (nNOS) and mitochondrial (mtNOS).[152,153]Each isoform is located in specific sub-cellular compartments which permit the physiological signaling of NO in various regulated cell signaling pathways that control smooth muscle relaxation, vasodilation, and regulation of blood pressure.[152] The multi-faceted physiological roles of these ROS and RNS will be assessed based on their sub-organellular production sites, which will establish their context in the pathophysiology of SCI (Table 3).[148]

Mitochondria

The mitochondria are widely thought to be the largest contributors toward the production of oxidative molecules within cells via the respiratory chain while generating ATP.[154] The mitochondria thus play a fundamental role as cell survival regulators by managing the balance between oxidative molecules and metabolic cell function. During oxidative phosphorylation, electrons are passed through a series of heme-containing complexes that help establish a proton gradient, which drives the chemiosmotic formation of ATP via the enzyme ATP-synthase. As this cell process is occurring, complexes I and III are believed to be the major producers of the ROS superoxide ($O_2\cdot^-$) by the one electron reduction of molecular oxygen (O_2), which is present in high levels in the mitochondrial matrix.[155] Multiple studies of isolated mitochondria have identified the redox reactions occurring at the active sites of complexes I and III and verified the generation of mitochondrial $O_2\cdot^-$ in the context of the respiratory chain.[156,157,158,159] $O_2\cdot^-$ is unable to pass through cell membranes and is relatively short-lived due to its reactivity and when present in physiological quantities is enzymatically converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) (Table 2).[160] SOD is able to react with $O_2\cdot^-$ because it contains a metal cofactor capable of accepting free electron. In the mitochondria, the predominant form of SOD contains manganese as its metal cofactor and is denoted Mn-SOD.[161] The enzyme-catalyzed reaction of $O_2\cdot^-$ to H_2O_2 occurs very efficiently, at an almost diffusion-limited rate of ~2 x 10^9 $M^{-1}\cdot s^{-1}$ which helps to keep basal levels of $O_2\cdot^-$ relatively low.[160,161] Likewise, the generation of H_2O_2 has been shown to exist in a steady-state concentration in cells in a study of catalase levels in isolated perfused rat livers in 1970 by Sies and Chance.[162] The levels of H_2O_2 are kept tightly regulated by a series of mitochondrial antioxidant processes driven mainly by classes of peroxiredoxins and glutathione peroxidases.[163,164] Tight regulation of H_2O_2 is necessary, as the molecule functions as an important signaling compound physiologically, demonstrated by its role in growth factor signaling, regulation of transcription factors, pathogen defense, cell survival, and proper protein folding.[164,165,166] Generally, these effects are mediated by the ability of H_2O_2 to oxidize thiol groups on cysteine residues that change the activity and roles of target proteins in signaling pathways.[167] Finally, mitochondria have been shown to contain NOS enzymes, iNOS and mtNOS, through studies of purified rat liver mitochondria, which are able to produce the RNS species nitric oxide

(NO).[153] One of the roles that NO plays in the mitochondria centers around a mechanism of inhibition of the respiratory chain, which then contributes to mitochondrial-regulated cell death.[153]

Endoplasmic Reticulum

The endoplasmic reticulum (ER) is the sub-organelle tasked with assembly and folding of proteins and serves as another important site for oxidative molecule activity and redox homeostasis.[168] The environment of the ER is unique, with the lumen maintaining a high oxidizing potential under physiological conditions to promote disulfide bond formation in unfolded polypeptides.[168] This oxidizing environment is created and maintained by the membrane-associated flavoprotein, ER oxidoreductin (Ero1).[169] Formation of disulfide bonds is critical for the proper folding of proteins and if there is a redox imbalance inside the ER lumen, errors in protein folding accumulate. High levels of misfolded proteins trigger ER stress signals to be transmitted into the cytoplasm and nucleus in the form of the unfolded protein response (UPR). The signaling cascades that are mediated by the UPR attempt to re-stabilize the redox homeostasis within the ER, but if it remains unbalanced for extended periods of time, the ER will signal for the initiation of apoptotic cell death cascades.[169] Ero1 functions to reduce O_2 by donating two electrons from its flavin cofactor, yielding H_2O_2.[169] H_2O_2 is subsequently scavenged by ER specific peroxiredoxin and glutathione peroxidase enzymes to maintain the redox homeostasis of the ER lumen.[170] Besides Ero1, studies have shown that the ER contains the enzymes nicotinamide adenine dinucleotide phosphate oxidase (NOX) and cytochrome P450 (cyto P450) that produce $O_2{\cdot}^-$ and, according to reviews by Laurindo et. al. in 2014 and Kim et. al. in 2009, play further roles in cell signaling during the UPR.[171,172]

Peroxisomes

Peroxisomes have been another key sub-organelle that have been evaluated in recent years for their role in cellular ROS/RNS metabolism.[173] This class of cell organelle plays a functional role in lipid metabolism, which includes many enzymes that produce ROS to help facilitate the breakdown of lipids.[174] Peroxisomes were originally discovered in a study by Christian de Duve in 1965 and characterized further by de Duve in 1966 as a cell organelle that contains at least one H_2O_2-producing enzyme and catalase, a specific H_2O_2 reducing enzyme.[175] Since de Duve's discoveries in the 1960s, additional studies have validated peroxisomes' capacity for producing ROS as an important aspect of normal metabolism.[174] The peroxisome is the site for many metabolic pathways that are regulated by a number of ROS/RNS producing enzymes, examples of which are highlighted in a review article by Antonenkov et. al. in 2010.[176] A few representative ROS producing enzymes are xanthine oxidase, which catalyzes the rate-limiting enzyme in purine catabolism and produces $O_2{\cdot}^-$, inducible nitric oxide synthase (iNOS), which catalyzes the production of NO from arginine, and Cu/Zn-SOD (an SOD with a copper-zinc metal cofactor), which forms H_2O_2 from

$O_2{\cdot}^-$.[176] Catalase serves as the major antioxidant enzyme in peroxisomes by catalyzing the reaction of H_2O_2 to H_2O and O_2.[174]

Immune Cells

The presence of ROS species in the cells of the immune system are derived from the NOX enzyme.[177] The discovery of the NOX family of enzymes in immune cells was the first time ROS were characterized in a physiological context. Biochemical analyses of phagocytosis by Sbarra et. al. in an experimental study of guinea pigs in 1959 demonstrated that ROS aids neutrophils in the phagocytosis of pathogens.[178] The activity of NOX is regulated by its catalytic subunit gp91phox, which becomes activated when it interacts with cytosolic subunits p22phox, p47phox, p40phox, and p67phox. Once the active complex is formed, NOX transfers electrons from NADPH to O_2, generating $O_2{\cdot}^-$ molecules.[179] Neutrophils and macrophages are then able to convert $O_2{\cdot}^-$ into hypochlorous acid (HOCl) using the enzyme myeloperoxidase.[180] HOCl is a strong oxidant and can mediate direct oxidative damage to cells and is naturally scavenged by organic compounds such as taurine and methionine, which rapidly react with HOCl to form nontoxic byproducts.[180,181,182] The generated ROS species of these immune cells subsequently interact and initiate cytotoxic destruction of pathogens.[183]

Table 3. Overview of Oxidative Molecules: Role in Physiology and Pathophysiology

Summary of the oxidative molecules involved in secondary spinal cord injury. *ER – Endoplasmic Reticulum, SOD – Superoxide Dismutase, MnSOD – Manganese Superoxide Dismutase, Cu/ZnSOD – Copper/Zinc Superoxide Dismutase, p.i. – Post-injury, GPx – Glutathione Peroxidase, Prx – Peroxiredoxin, GSH – Reduced Glutathione, GSSG – Oxidized Glutathione Disulfide, PUFA – Polyunsaturated Fatty Acids, cGMP – Cyclic Guanosine Monophosphate, VGCC- Voltage-Gated Calcium Channel, NOS – Nitric Oxide Synthase, iNOS – Inducible Nitric Oxide Synthase, eNOS – Endothelial Nitric Oxide Synthase, nNOS – Neuronal Nitric Oxide Synthase, NO_2^- – Nitrogen Dioxide, ETC – Electron Transport Chain, NO^- – Nitrate, L – Lipid, LP – Lipid Peroxidation, 4-HNE – 4-Hydroxynonenal*

Oxidative Molecules	***Physiological Location/Function***	***Antioxidants/ Defense Mechanisms***	***Biochemical Reactions***	***Pathophysiology in SCI***	***Time Course***
$O_2^{\cdot -}$ Superoxide)	**Location**: Cytoplasm[184], Immune Cells[185], ER[184], Mitochondria[184] **Function:** Byproduct of Many Enzyme-Catalyzed Reactions[145], Pathogen Defense[185]	**SOD: E**nzyme That Converts $2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$[161] (Two Classes are MnSOD and Cu/ZnSOD)[160]	$NO + O_2^{\cdot -} \rightarrow ONOO^-$[160] **Haber-Weiss Reaction**[186] **(Iron-Catalyzed)** $O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + OH\cdot + OH^-$	**Cytoplasm:** Elevated Levels of $O_2^{\cdot -}$ Oxidize and Release Catalytic Iron Triggering the Fenton/Haber-Weiss Reactions[186], $O_2^{\cdot -}$ Directly Reacts With NO to Form the Strong Oxidant $ONOO^-$[160]	0-12 Hours p.i.[187]
H_2O_2 (Hydrogen Peroxide)	**Location**: Cytoplasm[163], Mitochondria[163,167], Peroxisomes[163], ER[184], Extracellular Space[163] **Function:** Role in Growth Factor Signaling[165], Regulation of Transcription Factors[164], Pathogen Defense[166], Role in Cell Survival[166]	**Catalase:** Enzyme That Converts $H_2O_2 \rightarrow H_2O$ in Peroxisomes[163] **GPx:** Enzyme That Converts $2GSH + H_2O_2 \rightarrow 2H_2O + GSSG$[163] **Prx:** Enzyme That Converts $H_2O_2 \rightarrow H_2O$ in Mitochondria and Cytoplasm[163,164]	$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O$[180] **Fenton Reaction**[186] $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$ **Haber-Weiss Reaction**[186] **(Iron-Catalyzed)** $O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + OH\cdot + OH^-$	**Cytoplasm:** Conversion to OH· By Fenton/Haber-Weiss Reactions and Induction of Cell Death[188] **Nucleus:** Inactivation of transforming DNA[163]	0-11 Hours p.i.[189]
OH· (Hydroxyl Radical)	**Location**: Cytoplasm[163,184], Mitochondria[163,184], Nucleus[190] **Function**: Rapid Non-Specific Reactivity With Numerous Biomolecules (Lipids, DNA, Proteins)[191]	**Organic Compounds:** Glucose[192], Mannitol[193], Formate[194], Thiourea[194], and Dimethyl Sulfoxide[194] All Scavenge OH·	OH· + Lipids → Lipid Peroxidation[191] OH· + DNA → DNA Damage[191] OH· + Proteins → Protein Modifications[191]	**Nucleus:** Generation of OH· By Fenton/Haber-Weiss or Peroxynitrite Degradation Leads to Indirect Strand Breakage of DNA[190], Increases $[Ca^{2+}]_i$ Leading to Cell Death[195,196]	0-3 Hours p.i.[197]

Table 3. Overview of Oxidative Molecules: Role in Physiology and Pathophysiology Continued

Oxidative Molecules	*Physiological Location/Function*	*Antioxidants/ Defense Mechanisms*	*Biochemical Reactions*	*Pathophysiology in SCI*	*Time Course*
HClO (Hypochlorous Acid)	**Location**: Immune Cells[198] **Function**: Strong Oxidant Produced As Innate Immune Mechanism[180]	**Organic Compounds:** Taurine[182], Methionine[181] Scavenge HOCl	$HOCl$ + Proteins → Protein Modifications[183]	**Extracellular:** Generation of Aminoacyl Chloramines Leading to Cell Death or Growth Arrest[183]	0-12 Hours p.i.[198]
NO (Nitric Oxide)	**Location**: Cytoplasm[152], Immune Cells[199], Mitochondria[152] **Function:** Vasodilation Via cGMP[200], Regulates Gene Expression[201], Mediator of Angiogenesis[202], Wound Healing[202]	**Oxyhemoglobin:** Converts $NO \rightarrow NO^-$[200,203]	$NO + O_2^{\cdot -} \rightarrow ONOO^-$[160]	**Mitochondria:** Increased Production of NO[152], Inhibition of Cytochrome *c* Oxidase[152] **Cytoplasm:** Increased Production of NO Via Ca^{2+}-Dependent Upregulation of NOS (iNOS, eNOS, nNOS)[204]	0-2 Hours p.i.[205]
$ONOO^-$ (Peroxynitrite)	**Location:** Mitochondria[206], Immune Cells[206], Cytosol[207], Nucleus[200] **Function:** Strong Oxidant Reactions With Biomolecules (Lipids, DNA, Proteins)[200], Role in Growth Factor Signaling[200], Regulation of Transcription Factors[200], Inflammatory Response[200], Role in Cell Survival[200]	**Organic Compounds:** Uric Acid Scavenges $ONOO^-$[208] **Prx:** Enzyme That Converts $ONOO^- \rightarrow NO_2^-$[209]	$ONOO^- + CO_2 \rightarrow CO_3^{\cdot -} + \cdot NO_2$[210] $ONOO^- + H^+ \rightarrow OH\cdot + \cdot NO_2$[210] $ONOO^-$ + Lipids → Lipid Peroxidation[200] $ONOO^-$ + DNA → DNA Damage[200] $ONOO^-$ + Proteins → Protein Modification[200]	**Mitochondria:** Inactivation of Mn-SOD/GPx[209,211], Mediates Cell Death Pathways Via ETC Disruption and Cytochrome C Nitration[200,207], Formation of Secondary Strong Oxidants $CO_3^{\cdot -}/\cdot NO_2/OH\cdot$[210,212] **Nucleus:** Oxidative Damage of Nucleobases and Sugar-Phosphate Backbone Resulting in DNA Damage[200] **Membranes:** Triggers Lipid Peroxidation In PUFA Increasing Membrane Permeability[200]	0-3 Hours p.i.[205]

Table 3. Overview of Oxidative Molecules: Role in Physiology and Pathophysiology Continued

Oxidative Molecules	*Physiological Location/Function*	*Antioxidants/ Defense Mechanisms*	*Biochemical Reactions*	*Pathophysiology in SCI*	*Time Course*
Fe^{2+} (Ferrous)	**Location**: Cytoplasm[213], Red Blood Cells[214] **Function:** Facilitates Oxygen Transport by Hemoglobin in Fe^{3+} Form[214], Participates in Heme Enzyme-Related Reactions in Fe^{3+} Form[215]	**Ferritin:** Intracellular Protein That Stores Fe^{2+}[216] **Ceruloplasmin;** Ferroxidase That Converts $Fe^{2+} \rightarrow Fe^{3+}$[214]	**Fenton Reaction**[186] $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$ **Haber-Weiss Reaction**[186] **(Iron-Catalyzed)** $O_2\cdot^- + H_2O_2 \rightarrow O_2 + OH\cdot + OH^-$	**Cytoplasm:** Accumulation of Cytoplasmic Fe^{2+} Following Hemorrhage/Degradation of Hemoglobin Via Transferrin-Tf Receptor System[213], Generates $OH\cdot$ by Fenton/Haber-Weiss Reactions and Initiates Ferroptosis[213,217]	0-40 Minutes p.i.[218]
LOO·/LO· (Lipid Radicals)	**Location**: Cell Membrane[219] **Function:** 4-HNE Involved in Cellular Proliferation[220], Cell Differentiation[220], Cytoprotective Response[220]	**Organic Compounds:** Vitamin C and Vitamin E, Form Stable Vitamin Radicals[221] **GPx:** Reduction/Stabilization of Lipid Radicals[221]	**Initiation**[222] $L + ROS \rightarrow ROS\text{-}H + L\cdot$ **Propagation**[222] $L\cdot + O_2 \rightarrow LOO\cdot + O_2^-$ $LOO\cdot + L\text{-}H \rightarrow LOOH + L\cdot$ **Termination**[222] $2\ LOO\cdot \rightarrow LOO\text{-}OOL$ $LOO\text{-}OOL \rightarrow$ Non-Radical End Products	**Cytoplasm:** Lipid Radical Catalyzed Protein Carbonylation[223], **Membrane:** Increased Membrane Fluidity and Destruction of Lipid Bilayer[223], Acrolein and 4-HNE (Neurotoxic LP End Products) Cause Mitochondrial Respiratory Dysfunction[223]	0-12 Hours p.i.[224]

Oxidative Molecules: Role in SCI Neuroinflammation

Neuroinflammation, the process of endogenous CNS immune cell response to injury, interacts with peripheral inflammation and oxidative molecules to continue lesion destruction in SCI. While many important research studies have redefined the role of ROS and RNS as key regulators of many physiological pathways in redox homeostasis conditions, perturbations of their tight control can lead to significant damage. As diseased or injured tissue induces stress at the subcellular level, the balance between oxidative molecules and antioxidant enzymes begins to favor a “pro-oxidant” state that can cause damage to cells.[225,226] Such is the case in the sequence of secondary injury mechanisms of SCI, where many of the immediate pathological responses such as hemorrhagic necrosis, ischemia-reperfusion, inflammatory cell response, glutamate excitotoxicity, and ionic disturbances (Table 1) all contribute to tipping the redox balance towards an environment favoring the production of ROS and RNS. Effects of elevated $O_2\cdot^-$, which is one of the main initial ROS molecules that are produced due to disruption of cellular respiration, are multifaced, as the molecule can 1) lead to an increase in the labile iron pool by reacting with many subcellular heme-containing proteins, freeing Fe^{2+} 2) promote an increase in the SOD catalyzed conversion of $O_2\cdot^-$ to increase intracellular levels of H_2O_2, which based on its chemical nature can travel across cell membranes and participate in many inflammatory signal transduction pathways and 3) undergo reactions with other radical or non-radical species to generate highly reactive and strongly oxidizing molecules, such as $\cdot OH$ and $ONOO^-$ (Figure 3A).[184] Both $\cdot OH$ and $ONOO^-$ can induce high levels of neurodegeneration by causing DNA damage, protein oxidation and lipid radical formation. [196,227] A number of review articles and experimental studies, both *in vivo* and *in vitro*, have demonstrated presence of elevated levels and roles of oxidative molecules in SCI.[225,226,228,229,230,231,232] Besides the impact oxidative molecules have as direct inducers of damage, they also contribute to neuroinflammation in three key ways: 1) by triggering inflammation, 2) serving as mediators of inflammation, and 3) as effectors of inflammation.

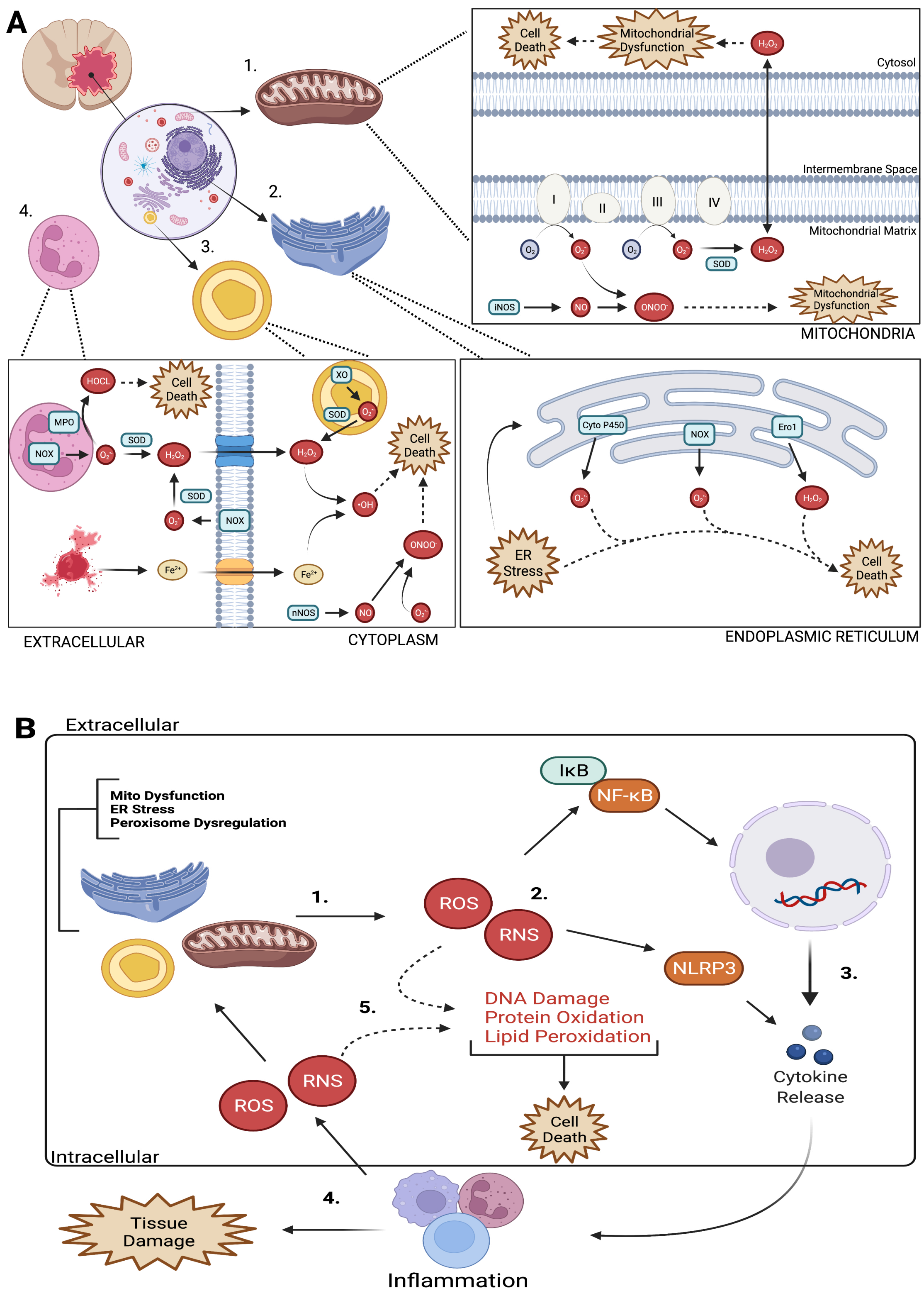

Figure 3. Oxidative Molecule Production in SCI Pathophysiology.

Figure 3A. Representative single cell from a lesion zone of traumatic spinal cord injury with identification of the key sub-organelle sites of production of oxidative molecules and their subsequent reactions. There is a dynamic interplay between many of these oxidative molecules, which function to exacerbate damage and contribute to apoptotic/necrotic cell death, prolonged inflammation, and increased size of lesion. 1) Mitochondrial $O_2\cdot^-$ production from complex I and complex III undergoes dismutation by the enzyme SOD to form H_2O_2, which can traverse the mitochondrial double membrane and enter the cytosol. NO is produced from the iNOS enzyme in the mitochondria under pathophysiological conditions, which can then react with $O_2\cdot^-$ to form $ONOO^-$. Mitochondrial dysfunction can arise from internal/external elevations in ROS/RNS species and results in cell death. 2) The endoplasmic reticulum contains a series of enzymes that produce $O_2\cdot^-$ (Cyto P450 and NOX) and H_2O_2 (Ero1). The production of these oxidative molecules in the ER is upregulated during ER stress, leading to cell death. 3) Peroxisomes are located in the cytoplasm and contain the enzymes XO and SOD, which produce $O_2\cdot^-$ and H_2O_2. The elevated levels of H_2O_2 then react with increased intracellular levels of Fe^2 via the Fenton/Haber-Weiss reactions to form ·OH, a strong oxidant that leads to stimulation of cell death pathways. Along with peroxisomes, nNOS enzymes are also located in the cytoplasm and produce NO, which can react with $O_2\cdot^-$ to form $ONOO^-$, contributing to cell death. 4) Immune cells contain enzymes that produce $O_2\cdot^-$ (NOX) and HOCl (MPO). Production of $O_2\cdot^-$ in the extracellular space gets converted to H_2O_2 via SOD, which then is transported into cells via aquaporin channels. HOCl is a strong oxidizing agent that can directly mediate cell death. Additionally, the extracellular space produces $O_2\cdot^-$ via plasma membrane NOX enzymes. Created with BioRender.com.

Figure 3B. Cellular targets of oxidative molecules and how they mediate increased damage in SCI. 1) Sub-organelle dysregulation, including mitochondrial disfunction, ER stress, and peroxisome dysregulation all contribute to increased ROS/RNS molecule production. 2) ROS/RNS molecules signal regulators of inflammation, including the NLRP3 inflammasome that directly signals cytokines and NF-κB, which is a transcription factor present in the cytosol and is negatively inhibiting by IκB. Release of IκB mediated by ROS/RNS molecules allows NF-κB to bind to DNA, leading to increased production of cytokines. 3) Cytokine release signals the activation and recruitment of inflammatory cells to the site of the lesion. 4) Inflammatory cells lead to increased tissue damage and the production of more ROS/RNS molecules, which further contribute to sub-organelle dysregulation. 5) ROS/RNS molecule accumulation leads to DNA damage, protein oxidation, and lipid peroxidation, which all serve to destabilize the cell and leads to cell death via necrotic and apoptotic pathways. Created with BioRender.com.

Oxidative Molecules: Triggers of Neuroinflammation

Oxidative molecules serve as triggers for neuroinflammation by initiating a number of signaling pathways that recruit inflammatory cells to the lesion. One of the most important and well-studied mechanisms of oxidative-induced neuroinflammatory signaling is through the transcription factor NF-κB (Figure 3B).[233] Typically, NF-κB is found in the cell cytoplasm and is in a complex form with the inhibitory protein IκB.[234] When activated, NF-κB is translocated to the nucleus and one of its roles includes transcriptional induction of pro-inflammatory cytokines.[235] Elevated levels of H_2O_2 have been shown in a number of studies to indirectly activate NF-κB by activating upstream kinases (IKK, NIK, Akt) which phosphorylate and release the IκB inhibitory protein.[233,236,237] Specifically, a study by Kim et. al (2009) provided evidence that H_2O_2 modulates NF-κB via activation of the PI3K/PTEN/Akt and NIK/IKK pathways.[237] ER stress, which can be induced by oxidative stress, has also been implicated in activation of the NF-κB through the PERK-mediated phosphorylation of eIF2α, which downregulates IκB.[233] Oxidative damage has also been shown to activate the NLRP3 inflammasome, a signaling protein that is known to convert pro-caspase 1 into active caspase 1, which subsequently activates IL-1β, an important pro-inflammatory cytokine.[238,239] Activation of the NLRP3 inflammasome has been addressed in reviews that highlight the oxidative-induced ER stress that is implicated in inflammatory disorders (Figure 3B).[239,240] A study by Shimada et. al. in 2012 identified a mitochondrial role in NLRP3 activation as well, showing that the oxidation of mitochondrial DNA in mice is an important signal for activation of the inflammasome.[241] In addition, $ONOO^-$ has been found to modulate several inflammatory pathways, which is reviewed extensively by Szabó et. al.[242] Included in the role of $ONOO^-$ in mediating inflammation following SCI is its upregulation of NF-κB, activation of the PI3K/Akt pathway, and enhanced production of pro-inflammatory mediators through the reduction of histone deacetylase 2 (HDAC2) activity.[242]

Oxidative Molecules: Mediators of Neuroinflammation

Once neuroinflammation is triggered in SCI, oxidative molecules continue to mediate the neuroinflammatory response through the continued upregulation of pro-inflammatory gene regulation, but also by helping direct and recruit peripheral inflammatory cells to the lesion. Studies of wound healing have shown that the oxidative molecules H_2O_2 and $O_2{\cdot}^-$ can stimulate chemotaxis and adhesion molecule expression.[243] A study by Loo et. al. demonstrated that there was an increased concentration of H_2O_2 at the site of neutrophil infiltration by using immunofluorescence staining in mice, positing a relationship between the NOX-dependent generation of $O_2{\cdot}^-$ and the subsequent SOD-catalyzed formation of H_2O_2 in the extracellular space.[244] Further studies have implicated an additional role of H_2O_2 in activating NF-κB, as the transcription factor also regulates the expression of pro-inflammatory adhesion molecules ICAM-1 and P-selectin.[245] The elevated expression of these activated adhesion molecules allows for recruitment and infiltration of circulating leukocytes through the endothelium, increasing the presence of inflammatory cells at the site of the lesion.[245] H_2O_2 has also been shown to generate platelet activating factor, which upregulates β_2 integrins on the cell membranes of leukocytes and allow the cells to dock on the classes of ICAM's and selectins.[245] Chemotaxis has been shown to further drive recruitment of inflammatory cells in studies of wound healing, which is mediated by H_2O_2 via activation of the redox sensor Lyn (an SFK).[243] As inflammatory cells are recruited to the site of the lesion, many of them continue the release of cytokines.[70] Cytokine release has been demonstrated in a study by Yang et. al. to increase mitochondrial and NADPH oxidase generated ROS in human RPE cells.[246] The compounding relationship between pro-inflammatory cytokines and oxidative molecules establishes a mediating effect that prolongs inflammatory damage at the site of the injury.[247]

Oxidative Molecules: Effectors of Neuroinflammation

As discussed previously, neuroinflammatory cells recruit further peripheral inflammatory cells. These neutrophils and macrophages can produce oxidative molecules that act as effectors which eliminate pathogens, but also can result in tissue injury if not regulated properly.[248] The production of ROS occurs through the enzymes NOX, which generate high levels of $O_2\cdot^-$ as part of the inflammatory response following its deployment to the lesion center.[249] Elevated levels of H_2O_2 are then formed via the dismutation of $O_2\cdot^-$ by extracellular SOD enzymes. $O_2\cdot^-$ and H_2O_2 are then rapidly released during phagocytosis or in response to inflammatory mediators, such as C5a and Interleukin-8 (IL-8) in an action that is termed “oxidative burst”. [249,250] The process of releasing large quantities of ROS is an important function of innate immune cells to effectively eliminate pathogens through combined efforts with proteases or through direct oxidative damage, but the high reactivity of these oxidative molecules have also been shown to lead to bystander tissue damage in overactive inflammatory responses.[248] In addition to the damage that is mediated by $O_2\cdot^-$ and H_2O_2, the granule-localized enzyme myeloperoxidase can also create a significant inflammatory effector molecule, HOCl. Production of HOCl has been shown to exacerbate injury given its strong neurotoxic oxidant properties that can exert its effects on a number of biomolecules (i.e., thiol groups, thioethers, amine groups, heme groups, iron-sulfur centers, nucleotides, and tryptophan).[198,251,252] The direct-mediated damage of these oxidative molecules by inflammatory markers contributes to the amplification of oxidative molecule production, inflammatory response, and cell death. Given the complex interaction between oxidative molecules and the neuroinflammatory reaction, key ROS/RNS species, $ONOO^-$ and H_2O_2, should be evaluated for their potential to be effectively targeted therapeutically in order to mitigate the neuroinflammatory response and reinstate proper repair pathways following traumatic SCI

THERAPEUTIC INTERVENTION DEVELOPMENT

Therapeutic Targets: Hydrogen Peroxide and Peroxynitrite

To date, there is no FDA-approved standardized anti-oxidative damage treatment for SCI. Experimental studies were identified and analyzed using search methods on Embase, which included the search terms: “spinal cord injury” AND “hydrogen peroxide” OR “peroxynitrite” AND “treatment.” Inclusion criteria used for the search query were studies published within the past five years. Manual methods were used to select studies that demonstrated therapeutic potential while targeting these specific candidate ROS/RNS molecules. Separate searches were conducted for hydrogen peroxide and peroxynitrite targeted therapies, which yielded thirteen and three results, respectively, with one article from each search meeting the aforementioned inclusion criteria. In contrast, two general search queries of “spinal cord injury” AND “antioxidant” (representative of studies researching the broad targeting of ROS/RNS molecules) yielded 211 results in the last five years. The small number of studies that resulted from these searches and the known role of these molecules in pathophysiology after traumatic SCI support targeting oxidative molecules as a focus of future research. The two studies of H_2O_2 and $ONOO^-$ were selected based on their demonstrated mechanistic potential in targeting endogenous aspect of the injury redox environment, which is an important therapeutic focus for consideration in treating pathological oxidative damage. These two representative studies identify an area of research that ought to be further expanded upon so that researchers and clinicians may limit oxidative and neuroinflammatory damage and improve functional recovery (Table 4).

Therapeutic Study: Hydrogen Peroxide

A 2017 study by Wang et. al. evaluated a potential therapeutic mechanism of methane-rich saline treatment in a spinal cord ischemia-reperfusion (IR) injury model in rats.[253] Methane has previously been reported to demonstrate anti-oxidant, anti-inflammatory, and anti-apoptotic activity in IR-induced injury to the intestine, liver, heart, abdominal skin flaps, and retina, but had not yet been studied in spinal cord IR.[253] Sprague-Dawley rats were divided into six experimental groups: sham, methane only, IR only, IR-methane, Si-methane, and Con-methane. Si-methane group rats received intrathecal pretreatment of nuclear factor erythroid 2-related factor (Nrf-2) siRNA once a day for 3 days prior to IR injury and con-siRNA followed the same protocol but were injected with control siRNA. siRNA knocks down Nrf-2 and was administered to confirm that methane-induced neuroprotection was due to activation of that anti-inflammatory transcription factor. IR injury was induced by inflating a balloon catheter at the site of the left subclavian artery for a period of 9 minutes. The experimental group that received IR and methane treatment were given doses of 10 ml/kg intraperitoneally 10 minutes post-injury.

Methane was shown to significantly increase the neuroprotective capabilities of the spinal cord following IR injury, mainly through the activation of the Nrf-2 pathway. This pathway plays a pivotal role in upregulation of the antioxidant response element (ARE) that activates many antioxidants and cytoprotective genes, including SOD, catalase, and heme oxygenase-1.[254] The researchers identified an increase in Nrf-2 protein expression and nuclear translocation following treatment, highlighting a potential mechanistic target for methane-rich saline. Furthermore, protein expression of HO-1, SOD, catalase, and GSH were all elevated in methane-treated rats when compared to injured rats. Neuronal apoptosis following ischemia, which was evaluated using TUNEL staining, demonstrated that methane treated rats had dramatically reduced apoptosis in their cells following IR injury compared to the other experimental groups. Methane was also shown to indirectly inhibit the expression of pro-inflammatory cytokines TNF-α and IL-1β by limiting NF-κB translocation to the nucleus, which was dependent on Nrf-2 expression mediated by methane. The identification of the antioxidative properties of methane in downregulating H_2O_2, along with other oxidative molecules, and inflammatory markers leading to enhanced neuroprotection clearly demonstrates the possibilities of targeting oxidative molecules in injury pathway. It is also important to note that H_2O_2 has a physiological role, when maintained at moderately elevated levels, to activate Nrf-2 which may point to a positive feedback loop and target for methane-induced Nrf-2 activation. By decreasing elevated levels of H_2O_2 in acute or chronic SCI, recruitment of the physiological roles of H_2O_2 may lead to further physiological activation of Nrf-2 and more appropriate mediation of antioxidative/anti-inflammatory events post-injury.

Despite promising results, the application of these findings are limited by several factors. First, although the study did not describe any notable side effects in the rats, it was conducted in a 72-hour period. Such a short observation window limits the understanding of the long-term impacts of methane treatment. Furthermore, there are limitations of this study's therapeutic delivery window. While methane was delivered to subjects 10 minutes post-injury, this timeframe is not attainable in pre-clinical or clinical settings for the treatment of SCI.[38]

Therapeutic Study: Peroxynitrite

A 2018 study by Khan et al. evaluated the impact of the impaired nitric oxide metabolome, and the therapeutic potential of exogenous S-nitrosoglutathione (GSNO) administration.[255] GSNO is an endogenous molecule that is produced by the reaction of NO with glutathione, serving as one of the enzymatic redox pathways of NO. Many biochemical studies have demonstrated that GSNO is directly involved in cell signaling through its promotion of the S-nitrosylation of several target proteins, including inhibition of PTEN which leads to Akt-mediated neurorepair.[256] Overall, Khan et al. (2018) focused on the reduced levels of GSNO present following spinal cord injury and aimed to validate a therapeutic method for exogenous delivery of this antioxidant enzyme.[255] Sprague-Dawley rats with induced contusion SCI were separated into three experimental groups: SCI only, SCI treated with GSNO, and sham controls. GSNO was administered intravenously at a dose of 0.05 mg/kg body weight two hours post-injury and then once daily for 14 days post-injury. Findings from this study demonstrated that exogenous delivery of GSNO improved motor function scores based on the significantly higher Beattie Basso and Bresnahan locomotor rating scores compared to SCI only rats.[257] They also observed decreased neuropathic pain in the GSNO-treated group, who had increased mechanical withdrawal latency compared to the SCI only group. Importantly, GSNO treatment was shown to decrease the levels of 3-nitrotyrosine, a marker of neuronal peroxynitrite damage. Levels of nNOS were significantly attenuated following GSNO treatment, pointing to a potential link between elevated activity of nNOS specific NO production and peroxynitrite formation. Ultimately, Khan et al. (2018) highlight how targeting peroxynitrite may increase functional recovery and decrease neuropathic pain.

While exogenous administration of GSNO was linked to functional improvement following SCI via downregulation of peroxynitrite generation, this preliminary mechanism-based experiment was limited by its short time window of SCI. The pathway explored in this study requires further evaluation for its therapeutic potential with longer time courses of SCI.

Table 4. Targeted Therapies for $ONOO^-$ and H_2O_2

Summary table that includes information on the validated studies for treatment of H_2O_2 and $ONOO^-$ in models of spinal cord injury. i.v. – intravenous, 3-NT – 3-nitrotyrosine, nNOS – neuronal nitric oxide synthase, i.p. – intraperitoneal, Nrf-2 – nuclear factor-erythroid factor 2-related factor 2, HO-1 – heme oxygenase-1, SOD – superoxide dismutase, GSSG – glutathione disulfide, 8-OHdG – 8-hydroxy-2' -deoxygunaosine, NF-κB – nuclear factor kappa B, TNF-α -- tumor necrosis factor-alpha, IL-1β -- interleukin-1β, ICAM-1 – intercellular adhesion molecule, IR – Ischemia-reperfusion

Author/Year	Intervention	Study Type	Molecular Target	Therapeutic Effects	Therapeutic Mechanism
Khan, M. 2018	S-nitrosoglutathione (GSNO): 0.05 mg/kg body weight i.v. 2 hours post-injury and once daily for 14 days post-injury	*In vivo*: Sprague-Dawley rat models of contusion SCI	$ONOO^-$	↑ Functional Recovery ↑ Neuroprotection ↓ Neuropathic pain	↓ nNOS activation ↓ 3-NT/α-II-spectrin levels
Wang, L. 2017	Methane-rich saline: 10 ml/kg i.p. once 10 mins post-injury	*In vivo*: Sprague-Dawley rat models of spinal cord IR injury	H_2O_2	↑ Neuroprotection ↑ BSCB stability ↓ Apoptosis	↑ Nrf-2 activation ↑ HO-1/SOD/Catalase/glutathione ↓ H_2O_2/ $O_2^{\cdot-}$/GSSG/MDA/8-OHdG/3-NT levels ↓ NF-κB/TNF-α/IL-1β/ICAM-1

Challenges to Antioxidant Therapy

ROS and RNS molecules have a critical role in the SI outcomes following SCI, particularly through their intricate relationship with neuroinflammation. The extensive involvement of ROS in the pathogenesis of SCI has prompted numerous research studies on the neuroprotective effects of various antioxidants (including bioactive compounds and enzymes) in animal models of SCI.[258] These studies acknowledge the importance of oxidative stress in the sequelae of SI, but there remain significant challenges in developing targeted therapeutic intervention of ROS and RNS molecules. Possible explanations for the difficulties in obtaining efficacious results using antioxidant therapies include the lack of specificity of antioxidants to the appropriate cellular tissue and their high reactivity and likely interference with both disease-triggering and physiological ROS levels. The challenges of devising a pharmacological treatment that can effectively be delivered to the source of pathophysiological ROS and RNS thus needs to be addressed in future experimental studies. The critical role that many oxidative molecules play in normal physiology, as well as their concerted mechanisms to maintain homeostasis, must be appreciated when designing a therapeutic intervention.[259,260] Therefore, activation of endogenous antioxidant response mechanisms and/or developing methods for precise delivery and release of antioxidant molecules have therapeutic potential for future mechanistic studies.

The therapeutic value of a precisely delivered antioxidant approach to SCI recovery is further emphasized by the results of Yu et. al. (2009). This study was the first to demonstrate that a drug-releasing polymer could offer neuroprotective effects for cell-based therapy implantation in acute SCI.[261] These researchers utilized a poly-lactic-*co*-glycolic acid (PLGA) scaffold that was seeded both with human neural stem cells (hNSCs) and manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP), a scavenger of $ONOO^-$. This delivery was neuroprotective based on *in vitro* and *in vivo* experiments that demonstrated via TUNEL assay that MnTBAP released from the PLGA scaffold reduced human NSC apoptosis in the SCI environment. This study also demonstrated increased signaling of cell survival pathways (Erk/p38 MAPK) by Western blot and immunocytochemistry analysis following treatment with MnTBAP.

CONCLUSION

SCI pathophysiology is characterized by SI cascades that contribute to further neurodegeneration and diminish functional recovery. Oxidative molecules are one of the major SI triggers, mediators and effectors, as demonstrated by their direct interaction with surrounding proteins, enzymes, receptors, channels, membranes, or nucleic acids, as well as their critical role in cell signaling and regulating other cell messengers, and involvement in neuroinflammation. Thus, therapeutic targeting of key ROS and RNS molecules will be important to mitigate secondary tissue lesions through blocking SI processes of neuroinflammation and cell/axon loss. Among ROS, H_2O_2 plays many key roles in cell signaling pathways when presenting in cells at steady-state, physiological levels, but it can initiate inflammatory signaling through NF-κB, NLRP3, and other cell signaling pathways under abnormal conditions such as SCI. It also reacts with Fe^{2+} to generate highly reactive $OH^{\cdot}$, which causes direct damage to all surrounding structures. Regarding RNS, $ONOO^-$ has been shown in numerous studies to cause direct damage to DNA, proteins, and lipids, leading to exacerbation of neuronal damage and the signaling of neuroinflammation. The therapeutic studies of H_2O_2 and $ONOO^-$ demonstrate the potential of targeting endogenous antioxidant mechanisms (Nrf-2 pathway and NO metabolome) for the mitigation of functional deficits. The targeting of oxidative species has the potential to reduce the cascades of neuroinflammation and redox damage that, when left untreated, manifest clinically as the continued expansion of the lesion.

The emergence of new antioxidant therapies (i.e., intra-thecal infusion/drug pumps, nanotech and synthetic material and polymer-based controlled drug release delivery, and cell-based therapies that contain antioxidative properties) and improving technologies in single cell sequencing (i.e., spatial transcriptomics, proteomics, genomics) hold promise for the development of more efficacious, precise medicine treatment to manage oxidative stress in SCI.

LIST OF JOURNAL ABBREVIATIONS

ACS... American Chemical Biology

Acta Neurochir...Acta Neurochirurgica

Acta Neuropathol. .... Acta Neuropathologica

Adv. Sci..Advanced Science

Alcohol Health Res. World.. Alcohol Health and Research World

Anat. Rec. A. Discov. Mol. Cell. Evol. Biol..

The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology

Ann. Neurol..Annals of Neurology

Annu. Rev. Biochem... Annual Review of Biochemistry

Annu. Rev. Neurosci... Annual Review of Neuroscience

Annu. Rev. Pharmacol. Toxicol............Annual Review of Pharmacology and Toxicology

Antioxid. Redox Signal.. Antioxidants and Redox Signaling

Arch. Biochem. Biophys.................................. Archives of Biochemistry and Biophysics

Arch. Neurol. .... Archives of Neurology

Arch. Phys. Med. Rehabil..................... Archives of Physical Medicine and Rehabilitation

Autoimmune Dis... Autoimmune Diseases

Auton. Neurosci. Basic Clin. .... Autonomic Neuroscience: Basic and Clinical

Biochem. Biophys. Res. Commun..........................Biochemical and Biophysical Research Communications

Biochem. J.. Biochemical Journal

Biochem. Soc. Trans. .... Biochemical Society Transactions

Biochim. Biophys. Acta. .... Biochimica et Biophysica Acta

BMC Neurosci. .... Biomed Central Neuroscience

Brain Res. .... Brain Research

Brain Res. Rev. .... Brain Research Reviews

Cell Death Dis. .... Cell Death Disease

Cell Transplant. .... Cell Transplantation

Cell. Mol. Life Sci. .... Cellular and Molecular Life Sciences

Chem. Rev. .... Chemical Reviews

Clin. Chim. Acta. .... Clinica Chimica Acta

Clin. Exp. Pharmacol. Physiol. . Clinical and Experimental Pharmacology and Physiology

Clin. Neuropharmacol. .... Clinical Neuropharmacology

Curr. Med. Chem. .... Current Medicinal Chemistry

Curr. Opin. Neurobiol. .... Current Opinions in Neurobiology

Curr. Opin. Toxicol. .... Current Opinions in Toxicology

EMBO. .... European Molecular Biology Organization

Eur. Heart J. .... European Heart Journal

Eur. J. Neurosci. .... European Journal of Neuroscience

Exp. Eye Res. .... Experimental Eye Research

Exp. Neurol. .... Experimental Neurology

F1000Prime Rep. .... F1000Prime Reports

FASEB. .... Federation of American Societies for Experimental Biology

FEBS .... Federation of European Biochemical Societies

Free Radic. Biol. Med. .... Free Radical Biology and Medicine

Free Radic. Res. .... Free Radical Research

Front. Aging Neurosci. .... Frontiers in Aging Neuroscience

Front. Biol. .... Frontiers in Biology

Front. Cell Dev. Biol. .... Frontiers in Cell and Developmental Biology

Front. Cell. Infect. Microbiol. .... Frontiers in Cellular and Infection Microbiology

Front. Cell. Neurosci. .... Frontiers in Cellular Neuroscience

Front. Immunol. .... Frontiers in Immunology

Front. Neurosci. .... Frontiers in Neuroscience

Front. Physiol. .... Frontiers in Physiology

Geriatr. Orthop. Surg. Rehabil. .... Geriatric Orthopaedic Surgery and Rehabilitation

Int. Anesthesiol. Clin. .... International Anesthesiology Clinics

Int. J. Mol. Sci. .... International Journal of Molecular Sciences

IRE Trans. Bio-Med. Electron. .... IRE Transactions on Bio-medical Electronics

J. Bioenerg. Biomembr. .... Journal of Bioenergetics and Biomembranes

J. Biol. Chem. .... Journal of Biological Chemistry

J. Bone Joint Surg. Am. .... Journal of Bone and Joint Surgery

J. Burns Wounds. .... Journal of Burns and Wounds

J. Cell Biol. .... Journal of Cell Biology

J. Cell Sci. .... Journal of Cell Science

J. Clin. Biochem. .... Clinical Biochemistry

J. Clin. Biochem. Nutr. ....Journal of Clinical Biochemistry and Nutrition

J. Clin. Invest. ....Journal of Clinical Investigation

J. Clin. Neurosci...Journal of Clinical Neuroscience

J. Comp. Neurol. .... The Journal of Comparative Neurology

J. Comp. Neurol. .... The Journal of Comparative Neurology

J. Cyclic Nucleotide Res. ....Journal of Cyclic Nucleotide Research

J. Gerontol...Journal of Gerontology

J. Immunol. ....Journal of Immunology

J. Lab. Clin. Med...Journal of Laboratory and Clinical Medicine

J. Nerv. Ment. Dis. ....The Journal of Nervous and Mental Disease

J. Neurochem. .... Journal of Neurochemistry

J. Neurocytol. .... Journal of Neurocytology

J. Neuroimmunol...Journal of Neuroimmunology

J. Neuroinflammation ....Journal of Neuroinflammation

J. Neuroinflammation. ....Journal of Neuroinflammation

J. Neurol. Sci.. Journal of Neurological Sciences

J. Neuropathol. Exp. Neurol. ......Journal of Neuropathology and Experimental Neurology

J. Neurosci... The Journal of Neuroscience

J. Neurosurg. .... Journal of Neurosurgery

J. Neurotrauma.. Journal of Neurotrauma

J. North Am. Spine Soc.. North American Spine Society Journal

J. Pharmacol. Exp. Ther. .... Journal of Pharmacology and Experimental Therapeutics

J. Spinal Cord Med. .... Journal of Spinal Cord Medicine

J. Spinal Disord. .... Journal of Spinal Disorders

JAAOS. .... The Journal of the American Academy of Orthopaedic Surgeons

JAMA .... Journal of the American Medical Association

Life Sci. .... Life Sciences

Mol. Cell. .... Molecular Cell

N. Engl. J. Med. .... New England Journal of Medicine

Nat. Neurosci. .... Nature Neuroscience

Nat. Rev. Dis. Primers .... Nature Reviews Disease Primers

Nat. Rev. Drug Discov. .... Nature Reviews Drug Discovery

Nat. Rev. Immunol. .... Nature Reviews Immunology

Nat. Rev. Mol. Cell Biol. .... Nature Reviews Molecular Cell Biology

Nat. Rev. Neurosci. .... Nature Reviews Neuroscience

Neural Regen. Res. .... Neural Regeneration Research

Neurobiol. Dis. .... Neurobiology of Disease

Neurochem. Res. .... Neurochemistry Research

Neurocrit. Care .... Neurocritical Care

Neurosci. Bull. .... Neuroscience Bulletin

Neurosci. Res. .... Journal of Neuroscience Research

Oxid. Med. Cell. Longev. .... Oxidative Medicine and Cellular Longevity

Pharmacol. Rev. .... Pharmacological Reviews

Pharmacol. Ther. .... Pharmacology and Therapeutics

Physiol. Rev. .... Physiological Reviews

PLOS ONE. .... Public Library of Science

Proc. Natl. Acad. Sci. ...... Proceedings of the National Academy of Sciences of the United States of America

Proc. Soc. Exp. Biol. Med. .... Experimental Biology and Medicine

Prog. Brain Res. .... Progress in Brain Research

R. Soc. Lond. .... Royal Society of London

Redox Biol. .... Redox Biology

Sci. Rep. .... Scientific Reports

Signal Transduct. Target. Ther. .... Signal Transduction and Targeted Therapy

Surg. Neurol. Int. .... Surgical Neurology International

Top. Spinal Cord Inj. Rehabil. .... Topics in Spinal Cord Injury Rehabilitation

Toxicol. Lett. .... Toxicology Letters

Toxicol. Pathol .... Toxicologic Pathology

Wien. Klin. Wochenschr. .... Wiener Klinische Wochenschrift

BIBLIOGRAPHY

1. World Health Organization. (2013). Spinal cord injury. [cited 2022 Jan 15] Available from: https://www.who.int/news-room/fact-sheets/detail/spinal-cord-injury.

2. National Spinal Cord Injury Statistical Center. (2018). Spinal Cord Injury Facts and Figures at a Glance.

3. Ahuja, C.S., Wilson, J.R., Nori, S., Kotter, M.R.N., Druschel, C., Curt, A., and Fehlings, M.G. (2017). Traumatic spinal cord injury. Nat. Rev. Dis. Primer 3, 1–21.

4. Chen, Y., Tang, Y., Vogel, L.C., and DeVivo, M.J. (2013). Causes of Spinal Cord Injury. Top. Spinal Cord Inj. Rehabil. 19, 1–8.

5. DeVivo, M.J., and Chen, Y. (2011). Trends in New Injuries, Prevalent Cases, and Aging With Spinal Cord Injury. Arch. Phys. Med. Rehabil. 92, 332–338.

6. Chen, Y., He, Y., and DeVivo, M.J. (2016). Changing Demographics and Injury Profile of New Traumatic Spinal Cord Injuries in the United States, 1972–2014. Arch. Phys. Med. Rehabil. 97, 1610–1619.

7. Ikpeze, T.C., and Mesfin, A. (2017). Spinal Cord Injury in the Geriatric Population: Risk Factors, Treatment Options, and Long-Term Management. Geriatr. Orthop. Surg. Rehabil. 8, 115–118.

8. Kirshblum, S.C., Burns, S.P., Biering-Sorensen, F., Donovan, W., Graves, D.E., Jha, A., Johansen, M., Jones, L., Krassioukov, A., Mulcahey, M., Schmidt-Read, M., and Waring, W. (2011). International standards for neurological classification of spinal cord injury (Revised 2011). J. Spinal Cord Med. 34, 535–546.

9. Mayo Clinic. (2021). Spinal cord injury - Symptoms and causes. Mayo Clin. [cited 2022 Jan 16] Available from: https://www.mayoclinic.org/diseases-conditions/spinal-cord-injury/symptoms-causes/syc-20377890.

10. Eldahan, K.C., and Rabchevsky, A.G. (2018). Autonomic Dysreflexia after Spinal Cord Injury: Systemic Pathophysiology and Methods of Management. Auton. Neurosci. Basic Clin. 209, 59–70.

11. Kabatas, S., Yu, D., He, X.D., Thatte, H.S., Benedict, D., Hepgul, K.T., Black, P.M., Sabharwal, S., and Teng, Y.D. (2008). Neural and anatomical abnormalities of the gastrointestinal system resulting from contusion spinal cord injury. Neuroscience 154, 1627–1638.

12. Pizzolato, C., Gunduz, M.A., Palipana, D., Wu, J., Grant, G., Hall, S., Dennison, R., Zafonte, R.D., Lloyd, D.G., and Teng, Y.D. (2021). Non-invasive approaches to functional recovery after spinal cord injury: Therapeutic targets and multimodal device interventions. Exp. Neurol. 339, 113612.

13. Hughes, J.T. (1988). The Edwin Smith Surgical Papyrus: an analysis of the first case reports of spinal cord injuries. Paraplegia 26, 71–82.

14. Donovan, W.H. (1994). Operative and nonoperative management of spinal cord injury. A review. Paraplegia 32, 375–388.

15. Landsteiner, K. (2001). [Agglutination phenomena of normal human blood]. Wien. Klin. Wochenschr. 113, 768–769.

16. Wiener, A.S. (1943). Genetic Theory of the Rh Blood Types. Proc. Soc. Exp. Biol. Med. 54, 316–319.

17. Röntgen, W.C. (1896). On a New Kind of Rays. Science 3, 227–231.

18. Oldendorf, W.H. (1961). Isolated flying spot detection of radiodensity discontinuities--displaying the internal structural pattern of a complex object. IRE Trans. Bio-Med. Electron. BME-8, 68–72.

19. Damadian, R. (1971). Tumor detection by nuclear magnetic resonance. Science 171, 1151–1153.

20. Harrington, P.R. (1962). Treatment of scoliosis. Correction and internal fixation by spine instrumentation. J. Bone Joint Surg. Am. 44-A, 591–610.

21. Teng, Y.D., and Zafonte, R.D. (2021). Prelude to the special issue on novel neurocircuit, cellular and molecular targets for developing functional rehabilitation therapies of neurotrauma. Exp. Neurol. 341, 113689.

22. Dumont, R.J., Okonkwo, D.O., Verma, S., Hurlbert, R.J., Boulos, P.T., Ellegala, D.B., and Dumont, A.S. (2001). Acute spinal cord injury, part I: pathophysiologic mechanisms. Clin. Neuropharmacol. 24, 254–264.

23. Allen, A.R. (1914). REMARKS ON THE HISTOPATHOLOGICAL CHANGES IN THE SPINAL CORD DUE TO IMPACT. AN EXPERIMENTAL STUDY. J. Nerv. Ment. Dis. 41, 141–147.

24. Tator, C.H., and Fehlings, M.G. (1991). Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J. Neurosurg. 75, 15–26.

25. Anwar, M.A., Al Shehabi, T.S., and Eid, A.H. (2016). Inflammogenesis of Secondary Spinal Cord Injury. Front. Cell. Neurosci. 10, 98.

26. Park, E., Velumian, A.A., and Fehlings, M.G. (2004). The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J. Neurotrauma 21, 754–774.

27. Rabchevsky, A.G., Michael, F.M., and Patel, S.P. (2020). Mitochondria focused neurotherapeutics for spinal cord injury. Exp. Neurol. 330, 113332.

28. Fan, B., Wei, Z., Yao, X., Shi, G., Cheng, X., Zhou, X., Zhou, H., Ning, G., Kong, X., and Feng, S. (2018). Microenvironment Imbalance of Spinal Cord Injury. Cell Transplant. 27, 853–866.

29. Beattie, M.S., Hermann, G.E., Rogers, R.C., and Bresnahan, J.C. (2002). Cell death in models of spinal cord injury. Prog. Brain Res. 137, 37–47.

30. Fatima, G., Sharma, V.P., Das, S.K., and Mahdi, A.A. (2015). Oxidative stress and antioxidative parameters in patients with spinal cord injury: implications in the pathogenesis of disease. Spinal Cord 53, 3–6.

31. Teng, Y.D. (2019). Functional Multipotency of Stem Cells and Recovery Neurobiology of Injured Spinal Cords. Cell Transplant. 28, 451–459.

32. Hall, E.D., and Braughler, J.M. (1982). Glucocorticoid mechanisms in acute spinal cord injury: a review and therapeutic rationale. Surg. Neurol. 18, 320–327.

33. Cheung, V., Hoshide, R., Bansal, V., Kasper, E., and Chen, C.C. (2015). Methylprednisolone in the management of spinal cord injuries: Lessons from randomized, controlled trials. Surg. Neurol. Int. 6, 142.

34. H, B., R, W., K, K., CashaSteven, and G, F. (2018). A Review of Clinical Trials in Spinal Cord Injury Including Biomarkers. J. Neurotrauma .

35. Bracken, M.B., Collins, W.F., Freeman, D.F., Shepard, M.J., Wagner, F.W., Silten, R.M., Hellenbrand, K.G., Ransohoff, J., Hunt, W.E., and Perot, P.L. (1984). Efficacy of methylprednisolone in acute spinal cord injury. JAMA 251, 45–52.

36. Bracken, M.B., Shepard, M.J., Collins, W.F., Holford, T.R., Young, W., Baskin, D.S., Eisenberg, H.M., Flamm, E., Leo-Summers, L., and Maroon, J. (1990). 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. 322, 1405–1411.

37. Bracken, M.B., Shepard, M.J., Holford, T.R., Leo-Summers, L., Aldrich, E.F., Fazl, M., Fehlings, M., Herr, D.L., Hitchon, P.W., Marshall, L.F., Nockels, R.P., Pascale, V., Perot, P.L., Piepmeier, J., Sonntag, V.K., Wagner, F., Wilberger, J.E., Winn, H.R., and Young, W. (1997). Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury. Results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial. National Acute Spinal Cord Injury Study. JAMA 277, 1597–1604.

38. Shank, C.D., Walters, B.C., and Hadley, M.N. (2019). Current Topics in the Management of Acute Traumatic Spinal Cord Injury. Neurocrit. Care 30, 261–271.

39. ([Date unknown]). What are the treatments for spinal cord injury (SCI)? https://www.nichd.nih.gov/ [cited 2022 Feb 14] Available from: https://www.nichd.nih.gov/health/topics/spinalinjury/conditioninfo/treatments.

40. Li, Y., Walker, C.L., Zhang, Y.P., Shields, C.B., and Xu, X.-M. (2014). Surgical decompression in acute spinal cord injury: A review of clinical evidence, animal model studies, and potential future directions of investigation. Front. Biol. 9, 127–136.

41. Badhiwala, J.H., Ahuja, C.S., and Fehlings, M.G. (2018). Time is spine: a review of translational advances in spinal cord injury: JNSPG 75th Anniversary Invited Review Article. J. Neurosurg. Spine 30, 1–18.

42. Tator, C.H., and Koyanagi, I. (1997). Vascular mechanisms in the pathophysiology of human spinal cord injury. J. Neurosurg. 86, 483–492.

43. Bartanusz, V., Jezova, D., Alajajian, B., and Digicaylioglu, M. (2011). The blood-spinal cord barrier: morphology and clinical implications. Ann. Neurol. 70, 194–206.

44. Boldin, C., Raith, J., Fankhauser, F., Haunschmid, C., Schwantzer, G., and Schweighofer, F. (2006). Predicting Neurologic Recovery in Cervical Spinal Cord Injury With Postoperative MR Imaging. Spine 31, 554–559.

45. Choo, A.M., Liu, J., Lam, C.K., Dvorak, M., Tetzlaff, W., and Oxland, T.R. (2007). Contusion, dislocation, and distraction: primary hemorrhage and membrane permeability in distinct mechanisms of spinal cord injury. J. Neurosurg. Spine 6, 255–266.

46. Ducker, T.B., Kindt, G.W., and Kempe, L.G. (1971). Pathological findings in acute experimental spinal cord trauma. J. Neurosurg. 35, 700–708.

47. Nelson, E., Gertz, S.D., Rennels, M.L., Ducker, T.B., and Blaumanis, O.R. (1977). Spinal cord injury. The role of vascular damage in the pathogenesis of central hemorrhagic necrosis. Arch. Neurol. 34, 332–333.

48. Guizar-Sahagun, G., Martinez-Cruz, A., Franco-Bourland, R.E., Cruz-García, E., Corona-Juarez, A., Diaz-Ruiz, A., Grijalva, I., Reyes-Alva, H.J., and Madrazo, I. (2017). Creation of an intramedullary cavity by hemorrhagic necrosis removal 24 h after spinal cord contusion in rats for eventual intralesional implantation of restorative materials. PLOS ONE 12, e0176105.

49. Cao, Y., Wu, T., yuan, Z., Li, D., Ni, S., Hu, J., and Lu, H. (2015). Three-dimensional imaging of microvasculature in the rat spinal cord following injury. Sci. Rep. 5, 12643.

50. Sadrzadeh, S.M., Anderson, D.K., Panter, S.S., Hallaway, P.E., and Eaton, J.W. (1987). Hemoglobin potentiates central nervous system damage. J. Clin. Invest. 79, 662–664.

51. Kalogeris, T., Baines, C.P., Krenz, M., and Korthuis, R.J. (2012). Chapter Six - Cell Biology of Ischemia/Reperfusion Injury., in: Jeon, K.W. (ed). *International Review of Cell and Molecular Biology*. Academic Press, pps. 229–317.

52. Roa, K.R., Donnenfeld, H., Chusid, J.G., and Valdez, S. (1982). Acute myelopathy secondary to spinal venous thrombosis. J. Neurol. Sci. 56, 107–113.

53. Yuan, X., Wu, Q., Tang, Y., Jing, Y., Li, Z., and Xiu, R. (2019). Systemic microcirculation dysfunction after low thoracic spinal cord injury in mice. Life Sci. 221, 47–55.

54. Zivin, J.A., DeGirolami, U., and Hurwitz, E.L. (1982). Spectrum of Neurological Deficits in Experimental CNS Ischemia: A Quantitative Study. Arch. Neurol. 39, 408–412.

55. Fried, L.C., and Goodkin, R. (1971). Microangiographic observations of the experimentally traumatized spinal cord. J. Neurosurg. 35, 709–714.

56. Tator, C.H. (1992). Hemodynamic issues and vascular factors in acute experimental spinal cord injury. J. Neurotrauma 9, 139–140; discussion 141.

57. Brown, A., Nabel, A., Oh, W., Etlinger, J.D., and Zeman, R.J. (2014). Perfusion imaging of spinal cord contusion: injury-induced blockade and partial reversal by β2-agonist treatment in rats. J. Neurosurg. Spine 20, 164–171.

58. Yang, C.-H., Quan, Z.-X., Wang, G.-J., He, T., Chen, Z.-Y., Li, Q.-C., Yang, J., and Wang, Q. (2022). Elevated intraspinal pressure in traumatic spinal cord injury is a promising therapeutic target. Neural Regen. Res. 17, 1703–1710.

59. Lemke, M., and Faden, A.I. (1990). Edema development and ion changes in rat spinal cord after impact trauma: injury dose-response studies. J. Neurotrauma 7, 41–54.

60. Němecek, S. t, Petr, R., Suba, P., Rozsival, V., and Mělka, O. (1977). Longitudinal extension of oedema in experimental spinal cord injury--evidence for two types of post-traumatic oedema. Acta Neurochir. (Wien) 37, 7–16.

61. Cho, N., Hachem, L.D., and Fehlings, M.G. (2017). Chapter 14 - Spinal Cord Edema After Spinal Cord Injury: From Pathogenesis to Management., in: Badaut, J., and Plesnila, N. (eds). *Brain Edema*. San Diego: Academic Press, pps. 261–275.

62. Yashon, D., Bingham, W.G., Faddoul, E.M., and Hunt, W.E. (1973). Edema of the spinal cord following experimental impact trauma. J. Neurosurg. 38, 693–697.

63. David, S., Zarruk, J.G., and Ghasemlou, N. (2012). Chapter Five - Inflammatory Pathways in Spinal Cord Injury., in: Goldberg, J.L., and Trakhtenberg, E.F. (eds). *International Review of Neurobiology*. Academic Press, pps. 127–152.

64. Ousman, S.S., and Kubes, P. (2012). Immune surveillance in the central nervous system. Nat. Neurosci. 15, 1096–1101.

65. Serhan, C.N., Chiang, N., and Van Dyke, T.E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat. Rev. Immunol. 8, 349–361.

66. Profyris, C., Cheema, S.S., Zang, D., Azari, M.F., Boyle, K., and Petratos, S. (2004). Degenerative and regenerative mechanisms governing spinal cord injury. Neurobiol. Dis. 15, 415–436.

67. Hellenbrand, D.J., Quinn, C.M., Piper, Z.J., Morehouse, C.N., Fixel, J.A., and Hanna, A.S. (2021). Inflammation after spinal cord injury: a review of the critical timeline of signaling cues and cellular infiltration. J. Neuroinflammation 18, 284.

68. Telegin, G.B., Chernov, A.S., Konovalov, N.A., Belogurov, A.A., Balmasova, I.P., and Gabibov, A.G. (2020). Cytokine Profile As a Marker of Cell Damage and Immune Dysfunction after Spinal Cord Injury. Acta Naturae 12, 92–101.

69. Yang, T., Dai, Y., Chen, G., and Cui, S. (2020). Dissecting the Dual Role of the Glial Scar and Scar-Forming Astrocytes in Spinal Cord Injury. Front. Cell. Neurosci. 14, 78.

70. Ren, H., Chen, X., Tian, M., Zhou, J., Ouyang, H., and Zhang, Z. (2018). Regulation of Inflammatory Cytokines for Spinal Cord Injury Repair Through Local Delivery of Therapeutic Agents. Adv. Sci. 5, 1800529.

71. Zhang, J.-M., and An, J. (2007). Cytokines, Inflammation and Pain. Int. Anesthesiol. Clin. 45, 27–37.

72. Pineau, I., and Lacroix, S. (2007). Proinflammatory cytokine synthesis in the injured mouse spinal cord: Multiphasic expression pattern and identification of the cell types involved. J. Comp. Neurol. 500, 267–285.

73. Yang, L., Jones, N.R., Blumbergs, P.C., Van Den Heuvel, C., Moore, E.J., Manavis, J., Sarvestani, G.T., and Ghabriel, M.N. (2005). Severity-dependent expression of pro-inflammatory cytokines in traumatic spinal cord injury in the rat. J. Clin. Neurosci. 12, 276–284.

74. Yang, L., Blumbergs, P.C., Jones, N.R., Manavis, J., Sarvestani, G.T., and Ghabriel, M.N. (2004). Early expression and cellular localization of proinflammatory cytokines interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in human traumatic spinal cord injury. Spine 29, 966–971.

75. Olson, J.K., and Miller, S.D. (2004). Microglia Initiate Central Nervous System Innate and Adaptive Immune Responses through Multiple TLRs. J. Immunol. 173, 3916–3924.

76. Martinez, F.O., and Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. 6, 13.

77. Fleming, J.C., Norenberg, M.D., Ramsay, D.A., Dekaban, G.A., Marcillo, A.E., Saenz, A.D., Pasquale-Styles, M., Dietrich, W.D., and Weaver, L.C.

(2006). The cellular inflammatory response in human spinal cords after injury. Brain 129, 3249–3269.

78. Beck, K.D., Nguyen, H.X., Galvan, M.D., Salazar, D.L., Woodruff, T.M., and Anderson, A.J. (2010). Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. Brain 133, 433–447.

79. Popovich, P.G., Guan, Z., McGaughy, V., Fisher, L., Hickey, W.F., and Basso, D.M. (2002). The neuropathological and behavioral consequences of intraspinal microglial/macrophage activation. J. Neuropathol. Exp. Neurol. 61, 623–633.

80. Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Münch, A.E., Chung, W.-S., Peterson, T.C., Wilton, D.K., Frouin, A., Napier, B.A., Panicker, N., Kumar, M., Buckwalter, M.S., Rowitch, D.H., Dawson, V.L., Dawson, T.M., Stevens, B., and Barres, B.A. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541, 481–487.

81. Okada, S., Hara, M., Kobayakawa, K., Matsumoto, Y., and Nakashima, Y. (2018). Astrocyte reactivity and astrogliosis after spinal cord injury. Neurosci. Res. 126, 39–43.

82. Paixão, S., and Klein, R. (2010). Neuron-astrocyte communication and synaptic plasticity. Curr. Opin. Neurobiol. 20, 466–473.

83. Schnell, L., Fearn, S., Klassen, H., Schwab, M.E., and Perry, V.H. (1999). Acute inflammatory responses to mechanical lesions in the CNS: differences between brain and spinal cord. Eur. J. Neurosci. 11, 3648–3658.

84. Perez, J.-C., Gerber, Y.N., and Perrin, F.E. (2021). Dynamic Diversity of Glial Response Among Species in Spinal Cord Injury. Front. Aging Neurosci. 13, 769548.

85. Yuan, Y.-M., and He, C. (2013). The glial scar in spinal cord injury and repair. Neurosci. Bull. 29, 421–435.

86. Wanner, I.B., Anderson, M.A., Song, B., Levine, J., Fernandez, A., Gray-Thompson, Z., Ao, Y., and Sofroniew, M.V. (2013). Glial Scar Borders Are Formed by Newly Proliferated, Elongated Astrocytes That Interact to Corral Inflammatory and Fibrotic Cells via STAT3-Dependent Mechanisms after Spinal Cord Injury. J. Neurosci. 33, 12870–12886.

87. Taoka, Y., Okajima, K., Uchiba, M., Murakami, K., Kushimoto, S., Johno, M., Naruo, M., Okabe, H., and Takatsuki, K. (1997). Role of neutrophils in spinal cord injury in the rat. Neuroscience 79, 1177–1182.

88. Popovich, P.G., Wei, P., and Stokes, B.T. (1997). Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. J. Comp. Neurol. 377, 443–464.

89. Nguyen, H.X., O'Barr, T.J., and Anderson, A.J. (2007). Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. J. Neurochem. 102, 900–912.

90. Hellenbrand, D.J., Quinn, C.M., Piper, Z.J., Morehouse, C.N., Fixel, J.A., and Hanna, A.S. (2021). Inflammation after spinal cord injury: a review of the critical timeline of signaling cues and cellular infiltration. J. Neuroinflammation 18, 284.

91. Neirinckx, V., Coste, C., Franzen, R., Gothot, A., Rogister, B., and Wislet, S. (2014). Neutrophil contribution to spinal cord injury and repair. J. Neuroinflammation 11, 150.

92. Shechter, R., London, A., Varol, C., Raposo, C., Cusimano, M., Yovel, G., Rolls, A., Mack, M., Pluchino, S., Martino, G., Jung, S., and Schwartz, M. (2009). Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. PLoS Med. 6, e1000113.

93. DiSabato, D., Quan, N., and Godbout, J.P. (2016). Neuroinflammation: The Devil is in the Details. J. Neurochem. 139, 136–153.

94. Zrzavy, T., Schwaiger, C., Wimmer, I., Berger, T., Bauer, J., Butovsky, O., Schwab, J.M., Lassmann, H., and Höftberger, R. (2020). Acute and non-resolving inflammation associate with oxidative injury after human spinal cord injury. Brain 144, 144–161.

95. Alexander, J.K., and Popovich, P.G. (2009). Neuroinflammation in spinal cord injury: therapeutic targets for neuroprotection and regeneration., in: Verhaagen, J., Hol, E.M., Huitenga, I., Wijnholds, J., Bergen, A.B., Boer, G.J., and Swaab, D.F. (eds). *Progress in Brain Research*. Elsevier, pps. 125–137.

96. Harrow-Mortelliti, M., Reddy, V., and Jimsheleishvili, G. (2022). Physiology, Spinal Cord., in: *StatPearls*. Treasure Island (FL): StatPearls Publishing.

97. (1997). The Principles of Nerve Cell Communication. Alcohol Health Res. World 21, 107–108.

98. Dingledine, R., Borges, K., Bowie, D., and Traynelis, S.F. (1999). The Glutamate Receptor Ion Channels. Pharmacol. Rev. 51, 7–62.

99. Li, S., and Stys, P.K. (2000). Mechanisms of Ionotropic Glutamate Receptor-Mediated Excitotoxicity in Isolated Spinal Cord White Matter. J. Neurosci. 20, 1190–1198.

100. Doble, A. (1999). The role of excitotoxicity in neurodegenerative disease: implications for therapy. Pharmacol. Ther. 81, 163–221.

101. Faden, A.I., and Simon, R.P. (1988). A potential role for excitotoxins in the pathophysiology of spinal cord injury. Ann. Neurol. 23, 623–626.

102. McAdoo, D.J., Xu, G.-Y., Robak, G., and Hughes, M.G. (1999). Changes in Amino Acid Concentrations over Time and Space around an Impact Injury and Their Diffusion Through the Rat Spinal Cord. Exp. Neurol. 159, 538–544.

103. Choi, D.W. (1988). Glutamate neurotoxicity and diseases of the nervous system. Neuron 1, 623–634.

104. Chan, P.H., and Fishman, R.A. (1980). Transient formation of superoxide radicals in polyunsaturated fatty acid-induced brain swelling. J. Neurochem. 35, 1004–1007.

105. James, N.D., Bartus, K., Grist, J., Bennett, D.L.H., McMahon, S.B., and Bradbury, E.J. (2011). Conduction Failure following Spinal Cord Injury: Functional and Anatomical Changes from Acute to Chronic Stages. J. Neurosci. 31, 18543–18555.

106. Waller, A.V., and Owen, R. (1851). Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. Abstr. Pap. Commun. R. Soc. Lond. 5, 924–925.

107. Vargas, M.E., and Barres, B.A. (2007). Why is Wallerian degeneration in the CNS so slow? Annu. Rev. Neurosci. 30, 153–179.

108. Rotshenker, S. (2011). Wallerian degeneration: the innate-immune response to traumatic nerve injury. J. Neuroinflammation 8, 109.

109. Stoll, G., Griffin, J.W., Li, C.Y., and Trapp, B.D. (1989). Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. J. Neurocytol. 18, 671–683.

110. Ludwin, S.K. (1990). Oligodendrocyte survival in Wallerian degeneration. Acta Neuropathol. (Berl.) 80, 184–191.

111. Popovich, P.G., and Hickey, W.F. (2001). Bone Marrow Chimeric Rats Reveal the Unique Distribution of Resident and Recruited Macrophages in the Contused Rat Spinal Cord. J. Neuropathol. Exp. Neurol. 60, 676–685.

112. Reichert, F., and Rotshenker, S. (1996). Deficient activation of microglia during optic nerve degeneration. J. Neuroimmunol. 70, 153–161.

113. Li, S., and Sheng, Z.-H. (2022). Energy matters: presynaptic metabolism and the maintenance of synaptic transmission. Nat. Rev. Neurosci. 23, 4–22.

114. Burke, K.J., and Bender, K.J. (2019). Modulation of Ion Channels in the Axon: Mechanisms and Function. Front. Cell. Neurosci. 13.

115. Clausen, M.V., Hilbers, F., and Poulsen, H. (2017). The Structure and Function of the Na,K-ATPase Isoforms in Health and Disease. Front. Physiol. 8.

116. Mata, A.M., and Sepúlveda, M.R. (2005). Calcium pumps in the central nervous system. Brain Res. Brain Res. Rev. 49, 398–405.

117. Sullivan, P.G., Krishnamurthy, S., Patel, S.P., Pandya, J.D., and Rabchevsky, A.G. (2007). Temporal characterization of mitochondrial bioenergetics after spinal cord injury. J. Neurotrauma 24, 991–999.

118. Zhao, X.-Y., Lu, M.-H., Yuan, D.-J., Xu, D.-E., Yao, P.-P., Ji, W.-L., Chen, H., Liu, W.-L., Yan, C.-X., Xia, Y.-Y., Li, S., Tao, J., and Ma, Q.-H. (2019). Mitochondrial Dysfunction in Neural Injury. Front. Neurosci. 13.

119. Crompton, M. (1999). The mitochondrial permeability transition pore and its role in cell death. Biochem. J. 341, 233–249.

120. Scholpa, N.E., and Schnellmann, R.G. (2017). Mitochondrial-Based Therapeutics for the Treatment of Spinal Cord Injury: Mitochondrial Biogenesis as a Potential Pharmacological Target. J. Pharmacol. Exp. Ther. 363, 303–313.

121. Sesso, A., Marques, M.M., Monteiro, M.M.T., Schumacher, R.I., Colquhoun, A., Belizário, J., Konno, S.N., Felix, T.B., Botelho, L.A.A., Santos, V.Z.C., Da Silva, G.R., Higuchi, M. de L., and Kawakami, J.T. (2004). Morphology of mitochondrial permeability transition: morphometric volumetry in apoptotic cells. Anat. Rec. A. Discov. Mol. Cell. Evol. Biol. 281, 1337–1351.

122. Simon, C.M., Sharif, S., Tan, R.P., and LaPlaca, M.C. (2009). Spinal Cord Contusion Causes Acute Plasma Membrane Damage. J. Neurotrauma 26, 563–574.

123. Liu, W.-M., Wu, J.-Y., Li, F.-C., and Chen, Q.-X. (2011). Ion channel blockers and spinal cord injury. J. Neurosci. Res. 89, 791–801.

124. Agrawal, S.K., and Fehlings, M.G. (1996). Mechanisms of secondary injury to spinal cord axons in vitro: role of Na+, Na(+)-K(+)-ATPase, the Na(+)-H+ exchanger, and the Na(+)-Ca2+ exchanger. J. Neurosci. Off. J. Soc. Neurosci. 16, 545–552.

125. Lemke, M., Demediuk, P., McIntosh, T.K., Vink, R., and Faden, A.I. (1987). Alterations in tissue Mg++, Na+ and spinal cord edema following impact trauma in rats. Biochem. Biophys. Res. Commun. 147, 1170–1175.

126. Chiu, S.Y., and Ritchie, J.M. (1980). Potassium channels in nodal and internodal axonal membrane of mammalian myelinated fibres. Nature 284, 170–171.

127. Nashmi, R., and Fehlings, M.G. (2001). Mechanisms of axonal dysfunction after spinal cord injury: with an emphasis on the role of voltage-gated potassium channels. Brain Res. Brain Res. Rev. 38, 165–191.

128. Cook, N.L., Corrigan, F., and van den Heuvel, C. (2011). The role of magnesium in CNS injury., in: Vink, R., and Nechifor, M. (eds). *Magnesium in the Central Nervous System*. Adelaide (AU): University of Adelaide Press.

129. Kahraman, S., Ozgurtas, T., Kayali, H., Atabey, C., Kutluay, T., and Timurkaynak, E. (2003). Monitoring of serum ionized magnesium in neurosurgical intensive care unit: preliminary results. Clin. Chim. Acta Int. J. Clin. Chem. 334, 211–215.

130. Schanne, F.A.X., Kane, A.B., Young, E.E., and Farber, J.L. (1979). Calcium Dependence of Toxic Cell Death: A Final Common Pathway. Science 206, 700–702.

131. Kwon, B.K., Tetzlaff, W., Grauer, J.N., Beiner, J., and Vaccaro, A.R. (2004). Pathophysiology and pharmacologic treatment of acute spinal cord injury. Spine J. Off. J. North Am. Spine Soc. 4, 451–464.

132. Emery, E., Aldana, P., Bunge, M.B., Puckett, W., Srinivasan, A., Keane, R.W., Bethea, J., and Levi, A.D. (1998). Apoptosis after traumatic human spinal cord injury. J. Neurosurg. 89, 911–920.

133. Elmore, S. (2007). Apoptosis: A Review of Programmed Cell Death. Toxicol. Pathol. 35, 495–516.

134. Yong, C., Arnold, P.M., Zoubine, M.N., Citron, B.A., Watanabe, I., Berman, N.E., and Festoff, B.W. (1998). Apoptosis in cellular compartments of rat spinal cord after severe contusion injury. J. Neurotrauma 15, 459–472.

135. Liu, X.Z., Xu, X.M., Hu, R., Du, C., Zhang, S.X., McDonald, J.W., Dong, H.X., Wu, Y.J., Fan, G.S., Jacquin, M.F., Hsu, C.Y., and Choi, D.W. (1997). Neuronal and glial apoptosis after traumatic spinal cord injury. J. Neurosci. Off. J. Soc. Neurosci. 17, 5395–5406.

136. Kato, H., Kanellopoulos, G.K., Matsuo, S., Wu, Y.J., Jacquin, M.F., Hsu, C.Y., Kouchoukos, N.T., and Choi, D.W. (1997). Neuronal apoptosis and necrosis following spinal cord ischemia in the rat. Exp. Neurol. 148, 464–474.

137. Visavadiya, N.P., Patel, S.P., VanRooyen, J.L., Sullivan, P.G., and Rabchevsky, A.G. (2016). Cellular and subcellular oxidative stress parameters following severe spinal cord injury. Redox Biol. 8, 59–67.

138. Lewén, A., Matz, P., and Chan, P.H. (2000). Free radical pathways in CNS injury. J. Neurotrauma 17, 871–890.

139. Dizdaroglu, M., and Jaruga, P. (2012). Mechanisms of free radical-induced damage to DNA. Free Radic. Res. 46, 382–419.

140. Bains, M., and Hall, E.D. (2012). Antioxidant therapies in traumatic brain and spinal cord injury. Biochim. Biophys. Acta 1822, 675–684.

141. Phaniendra, A., Jestadi, D.B., and Periyasamy, L. (2015). Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J. Clin. Biochem. 30, 11–26.

142. Liu, D., Ling, X., Wen, J., and Liu, J. (2000). The Role of Reactive Nitrogen Species in Secondary Spinal Cord Injury. J. Neurochem. 75, 2144–2154.

143. Christie, S.D., Comeau, B., Myers, T., Sadi, D., Purdy, M., and Mendez, I. (2008). Duration of lipid peroxidation after acute spinal cord injury in rats and the effect of methylprednisolone. Neurosurg. Focus 25, E5.

144. Zimmerman, J.J., von Saint André-von Arnim, A., and McLaughlin, J. (2011). Chapter 74 - Cellular Respiration., in: Fuhrman, B.P., and Zimmerman, J.J. (eds). *Pediatric Critical Care (Fourth Edition)*. Saint Louis: Mosby, pps. 1058–1072.

145. Holmström, K.M., and Finkel, T. (2014). Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat. Rev. Mol. Cell Biol. 15, 411–421.

146. Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.

147. Lambeth, J.D. (2007). Nox enzymes, ROS, and chronic disease: An example of antagonistic pleiotropy. Free Radic. Biol. Med. 43, 332–347.

148. Sies, H., and Jones, D.P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat. Rev. Mol. Cell Biol. 21, 363–383.

149. Yoboue, E.D., Sitia, R., and Simmen, T. (2018). Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. Cell Death Dis. 9, 1–14.

150. Marsh, N., and Marsh, A. (2000). A Short History Of Nitroglycerine And Nitric Oxide In Pharmacology And Physiology. Clin. Exp. Pharmacol. Physiol. 27, 313–319.

151. Katsuki, S., Arnold, W., Mittal, C., and Murad, F. (1977). Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. J. Cyclic Nucleotide Res. 3, 23–35.

152. Förstermann, U., and Sessa, W.C. (2012). Nitric oxide synthases: regulation and function. Eur. Heart J. 33, 829–837.

153. Giulivi, C. (2003). Characterization and function of mitochondrial nitric-oxide synthase. Free Radic. Biol. Med. 34, 397–408.

154. Murphy, M.P. (2009). How mitochondria produce reactive oxygen species. Biochem. J. 417, 1–13.

155. Kehrer, J.P., Robertson, J.D., and Smith, C.V. (2010). Free Radicals and Reactive Oxygen Species., in: McQueen, C.A. (ed). *Comprehensive Toxicology (Second Edition)*. Oxford: Elsevier, pps. 277–307.

156. Kussmaul, L., and Hirst, J. (2006). The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. Proc. Natl. Acad. Sci. U. S. A. 103, 7607–7612.

157. Hirst, J., King, M.S., and Pryde, K.R. (2008). The production of reactive oxygen species by complex I. Biochem. Soc. Trans. 36, 976–980.

158. Turrens, J.F., Alexandre, A., and Lehninger, A.L. (1985). Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. Arch. Biochem. Biophys. 237, 408–414.

159. Chen, Q., Vazquez, E.J., Moghaddas, S., Hoppel, C.L., and Lesnefsky, E.J. (2003). Production of reactive oxygen species by mitochondria: central role of complex III. J. Biol. Chem. 278, 36027–36031.

160. Wang, Y., Branicky, R., Noë, A., and Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J. Cell Biol. 217, 1915–1928.

161. Fridovich, I. (1975). Superoxide dismutases. Annu. Rev. Biochem. 44, 147–159.

162. Sies, H., and Chance, B. (1970). The steady state level of catalase compound I in isolated hemoglobin-free perfused rat liver. FEBS Lett. 11, 172–176.

163. Chance, B., Sies, H., and Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59, 527–605.

164. Espinosa-Diez, C., Miguel, V., Mennerich, D., Kietzmann, T., Sánchez-Pérez, P., Cadenas, S., and Lamas, S. (2015). Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol. 6, 183–197.

165. Finkel, T. (2011). Signal transduction by reactive oxygen species. J. Cell Biol. 194, 7–15.

166. Stone, J.R., and Yang, S. (2006). Hydrogen peroxide: a signaling messenger. Antioxid. Redox Signal. 8, 243–270.

167. Sena, L.A., and Chandel, N.S. (2012). Physiological roles of mitochondrial reactive oxygen species. Mol. Cell 48, 158–167.

168. Shimizu, Y., and Hendershot, L.M. (2007). Organization of the Functions and Components of the Endoplasmic Reticulum., in: Csermely, P., and Vígh, L. (eds). *Molecular Aspects of the Stress Response: Chaperones, Membranes and Networks*. New York, NY: Springer, pps. 37–46.

169. Sevier, C.S., and Kaiser, C.A. (2008). Ero1 and redox homeostasis in the endoplasmic reticulum. Biochim. Biophys. Acta BBA - Mol. Cell Res. 1783, 549–556.

170. Yoboue, E.D., Sitia, R., and Simmen, T. (2018). Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. Cell Death Dis. 9, 1–14.

171. Laurindo, F.R.M., Araujo, T.L.S., and Abrahão, T.B. (2014). Nox NADPH oxidases and the endoplasmic reticulum. Antioxid. Redox Signal. 20, 2755–2775.

172. Kim, H.-R., Lee, G.-H., Cho, E.Y., Chae, S.-W., Ahn, T., and Chae, H.-J. (2009). Bax inhibitor 1 regulates ER-stress-induced ROS accumulation through the regulation of cytochrome P450 2E1. J. Cell Sci. 122, 1126–1133.

173. Sandalio, L.M., Rodríguez-Serrano, M., Romero-Puertas, M.C., and del Río, L.A. (2013). Role of Peroxisomes as a Source of Reactive Oxygen Species (ROS) Signaling Molecules., in: del Río, L.A. (ed). *Peroxisomes and Their Key Role in Cellular Signaling and Metabolism*. Dordrecht: Springer Netherlands, pps. 231–255.

174. Fransen, M., Nordgren, M., Wang, B., and Apanasets, O. (2012). Role of peroxisomes in ROS/RNS-metabolism: Implications for human disease. Biochim. Biophys. Acta BBA - Mol. Basis Dis. 1822, 1363–1373.

175. De Duve, C., and Baudhuin, P. (1966). Peroxisomes (microbodies and related particles). Physiol. Rev. 46, 323–357.

176. Antonenkov, V.D., Grunau, S., Ohlmeier, S., and Hiltunen, J.K. (2010). Peroxisomes are oxidative organelles. Antioxid. Redox Signal. 13, 525–537.

177. Tavassolifar, M. javad, Vodjgani, M., Salehi, Z., and Izad, M. (2020). The Influence of Reactive Oxygen Species in the Immune System and Pathogenesis of Multiple Sclerosis. Autoimmune Dis. 2020, 5793817.

178. Sbarra, A.J., and Karnovsky, M.L. (1959). The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. J. Biol. Chem. 234, 1355–1362.

179. Lambeth, J.D. (2004). NOX enzymes and the biology of reactive oxygen. Nat. Rev. Immunol. 4, 181–189.

180. Wang, L., Bassiri, M., Najafi, R., Najafi, K., Yang, J., Khosrovi, B., Hwong, W., Barati, E., Belisle, B., Celeri, C., and Robson, M. (2007). Hypochlorous Acid as a Potential Wound Care Agent. J. Burns Wounds 6, e5.

181. Weiss, S.J. (1989). Tissue destruction by neutrophils. N. Engl. J. Med. 320, 365–376.

182. Klamt, F., and Shacter, E. (2005). Taurine chloramine, an oxidant derived from neutrophils, induces apoptosis in human B lymphoma cells through mitochondrial damage. J. Biol. Chem. 280, 21346–21352.

183. Englert, R.P., and Shacter, E. (2002). Distinct modes of cell death induced by different reactive oxygen species: amino acyl chloramines mediate hypochlorous acid-induced apoptosis. J. Biol. Chem. 277, 20518–20526.

184. Indo, H.P., Yen, H.-C., Nakanishi, I., Matsumoto, K., Tamura, M., Nagano, Y., Matsui, H., Gusev, O., Cornette, R., Okuda, T., Minamiyama, Y., Ichikawa, H., Suenaga, S., Oki, M., Sato, T., Ozawa, T., Clair, D.K.St., and Majima, H.J. (2015). A mitochondrial superoxide theory for oxidative stress diseases and aging. J. Clin. Biochem. Nutr. 56, 1–7.

185. Vaziri, N.D., Lee, Y.-S., Lin, C.-Y., Lin, V.W., and Sindhu, R.K. (2004). NAD(P)H oxidase, superoxide dismutase, catalase, glutathione peroxidase and nitric oxide synthase expression in subacute spinal cord injury. Brain Res. 995, 76–83.

186. Kehrer, J.P. (2000). The Haber–Weiss reaction and mechanisms of toxicity. Toxicology 149, 43–50.

187. Liu, D., Sybert, T.E., Qian, H., and Liu, J. (1998). Superoxide production after spinal injury detected by microperfusion of cytochrome c. Free Radic. Biol. Med. 25, 298–304.

188. Liu, D., and Bao, F. (2015). Hydrogen peroxide administered into the rat spinal cord at the level elevated by contusion spinal cord injury oxidizes proteins, DNA and membrane phospholipids, and induces cell death: attenuation by a metalloporphyrin. Neuroscience 285, 81–96.

189. Liu, D., Liu, J., and Wen, J. (1999). Elevation of hydrogen peroxide after spinal cord injury detected by using the Fenton reaction. Free Radic. Biol. Med. 27, 478–482.

190. Balasubramanian, B., Pogozelski, W.K., and Tullius, T.D. (1998). DNA strand breaking by the hydroxyl radical is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone. Proc. Natl. Acad. Sci. U. S. A. 95, 9738–9743.

191. Freinbichler, W., Colivicchi, M.A., Stefanini, C., Bianchi, L., Ballini, C., Misini, B., Weinberger, P., Linert, W., Varešlija, D., Tipton, K.F., and Della Corte, L. (2011). Highly reactive oxygen species: detection, formation, and possible functions. Cell. Mol. Life Sci. 68, 2067–2079.

192. Sagone, A.L., Greenwald, J., Kraut, E.H., Bianchine, J., and Singh, D. (1983). Glucose: a role as a free radical scavenger in biological systems. J. Lab. Clin. Med. 101, 97–104.

193. Desesso, J.M., Scialli, A.R., and Goeringer, G.C. (1994). D-mannitol, a specific hydroxyl free radical scavenger, reduces the developmental toxicity of hydroxyurea in rabbits. Teratology 49, 248–259.

194. Bektaşoğlu, B., Esin Celik, S., Ozyürek, M., Güçlü, K., and Apak, R. (2006). Novel hydroxyl radical scavenging antioxidant activity assay for water-soluble antioxidants using a modified CUPRAC method. Biochem. Biophys. Res. Commun. 345, 1194–1200.

195. Ishii, M., Shimizu, S., Hara, Y., Hagiwara, T., Miyazaki, A., Mori, Y., and Kiuchi, Y. (2006). Intracellular-produced hydroxyl radical mediates H2O2-induced Ca2+ influx and cell death in rat β-cell line RIN-5F. Cell Calcium 39, 487–494.

196. Bao, F., and Liu, D. (2004). Hydroxyl radicals generated in the rat spinal cord at the level produced by impact injury induce cell death by necrosis and apoptosis: protection by a metalloporphyrin. Neuroscience 126, 285–295.

197. Liu, D., Liu, J., Sun, D., and Wen, J. (2004). The time course of hydroxyl radical formation following spinal cord injury: the possible role of the iron-catalyzed Haber-Weiss reaction. J. Neurotrauma 21, 805–816.

198. Kubota, K., Saiwai, H., Kumamaru, H., Maeda, T., Ohkawa, Y., Aratani, Y., Nagano, T., Iwamoto, Y., and Okada, S. (2012). Myeloperoxidase exacerbates secondary injury by generating highly reactive oxygen species

and mediating neutrophil recruitment in experimental spinal cord injury. Spine 37, 1363–1369.

199. Marsala, J., Orendácová, J., Lukácová, N., and Vanický, I. (2007). Traumatic injury of the spinal cord and nitric oxide. Prog. Brain Res. 161, 171–183.

200. PACHER, P., BECKMAN, J.S., and LIAUDET, L. (2007). Nitric Oxide and Peroxynitrite in Health and Disease. Physiol. Rev. 87, 315–424.

201. Gudi, T., Hong, G.K., Vaandrager, A.B., Lohmann, S.M., and Pilz, R.B. (1999). Nitric oxide and cGMP regulate gene expression in neuronal and glial cells by activating type II cGMP-dependent protein kinase. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 13, 2143–2152.

202. Hirst, D.G., and Robson, T. (2011). Nitric Oxide Physiology and Pathology., in: McCarthy, H.O., and Coulter, J.A. (eds). *Nitric Oxide: Methods and Protocols*. Totowa, NJ: Humana Press, pps. 1–13.

203. Azarov, I., Huang, K.T., Basu, S., Gladwin, M.T., Hogg, N., and Kim-Shapiro, D.B. (2005). Nitric oxide scavenging by red blood cells as a function of hematocrit and oxygenation. J. Biol. Chem. 280, 39024–39032.

204. Conti, A., Miscusi, M., Cardali, S., Germanò, A., Suzuki, H., Cuzzocrea, S., and Tomasello, F. (2007). Nitric oxide in the injured spinal cord: synthases cross-talk, oxidative stress and inflammation. Brain Res. Rev. 54, 205–218.

205. Liu, D., Ling, X., Wen, J., and Liu, J. (2000). The Role of Reactive Nitrogen Species in Secondary Spinal Cord Injury. J. Neurochem. 75, 2144–2154.

206. Ferrer-Sueta, G., and Radi, R. (2009). Chemical Biology of Peroxynitrite: Kinetics, Diffusion, and Radicals. ACS Chem. Biol. 4, 161–177.

207. Virág, L., Szabó, É., Gergely, P., and Szabó, C. (2003). Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. Toxicol. Lett. 140–141, 113–124.

208. Hooper, D.C., Scott, G.S., Zborek, A., Mikheeva, T., Kean, R.B., Koprowski, H., and Spitsin, S.V. (2000). Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood–CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis. FASEB J. 14, 691–698.

209. Dubuisson, M., Vander Stricht, D., Clippe, A., Etienne, F., Nauser, T., Kissner, R., Koppenol, W.H., Rees, J.-F., and Knoops, B. (2004). Human peroxiredoxin 5 is a peroxynitrite reductase. FEBS Lett. 571, 161–165.

210. Radi, R. (2013). Peroxynitrite, a Stealthy Biological Oxidant. J. Biol. Chem. 288, 26464–26472.

211. Padmaja, S., Squadrito, G.L., and Pryor, W.A. (1998). Inactivation of Glutathione Peroxidase by Peroxynitrite. Arch. Biochem. Biophys. 349, 1–6.

212. Augusto, O., Bonini, M.G., Amanso, A.M., Linares, E., Santos, C.C.X., and De Menezes, S.L. (2002). Nitrogen dioxide and carbonate radical anion: two emerging radicals in biology. Free Radic. Biol. Med. 32, 841–859.

213. Hu, X., Xu, Y., Xu, H., Jin, C., Zhang, H., Su, H., Li, Y., Zhou, K., and Ni, W. (2021). Progress in Understanding Ferroptosis and Its Targeting for Therapeutic Benefits in Traumatic Brain and Spinal Cord Injuries. Front. Cell Dev. Biol. 9.

214. Rathore, K.I., Kerr, B.J., Redensek, A., López-Vales, R., Jeong, S.Y., Ponka, P., and David, S. (2008). Ceruloplasmin Protects Injured Spinal Cord from Iron-Mediated Oxidative Damage. J. Neurosci. 28, 12736–12747.

215. Crichton, R. (2016). The Essential Role of Iron in Biology., in: *Iron Metabolism: From Molecular Mechanisms to Clinical Consequences*, Fourth. John Wiley & Sons, Ltd, pps. 22–70.

216. Balla, G., Jacob, H.S., Balla, J., Rosenberg, M., Nath, K., Apple, F., Eaton, J.W., and Vercellotti, G.M. (1992). Ferritin: a cytoprotective antioxidant strategem of endothelium. J. Biol. Chem. 267, 18148–18153.

217. Wan, J., Ren, H., and Wang, J. (2019). Iron toxicity, lipid peroxidation and ferroptosis after intracerebral haemorrhage. Stroke Vasc. Neurol. 4.

218. Liu, D., Liu, J., Sun, D., Alcock, N.W., and Wen, J. (2003). Spinal cord injury increases iron levels: catalytic production of hydroxyl radicals. Free Radic. Biol. Med. 34, 64–71.

219. Ayala, A., Muñoz, M.F., and Argüelles, S. (2014). Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid. Med. Cell. Longev. 2014, 360438.

220. Forman, H.J., Fukuto, J.M., Miller, T., Zhang, H., Rinna, A., and Levy, S. (2008). The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. Arch. Biochem. Biophys. 477, 183–195.

221. Traber, M.G., and Stevens, J.F. (2011). Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radic. Biol. Med. 51, 1000–1013.

222. Yin, H., Xu, L., and Porter, N.A. (2011). Free Radical Lipid Peroxidation: Mechanisms and Analysis. Chem. Rev. 111, 5944–5972.

223. Hall, E.D., Wang, J.A., Bosken, J.M., and Singh, I.N. (2016). Lipid Peroxidation in Brain or Spinal Cord Mitochondria After Injury. J. Bioenerg. Biomembr. 48, 169–174.

224. Christie, S.D., Comeau, B., Myers, T., Sadi, D., Purdy, M., and Mendez, I. (2008). Duration of lipid peroxidation after acute spinal cord injury in rats and the effect of methylprednisolone: Laboratory investigation. Neurosurg. Focus 25, E5.

225. Bains, M., and Hall, E.D. (2012). Antioxidant therapies in traumatic brain and spinal cord injury. Biochim. Biophys. Acta 1822, 675–684.

226. Hall, E.D. (2011). Antioxidant Therapies for Acute Spinal Cord Injury. Neurotherapeutics 8, 152–167.

227. Carrico, K.M., Vaishnav, R., and Hall, E.D. (2009). Temporal and Spatial Dynamics of Peroxynitrite-Induced Oxidative Damage after Spinal Cord Contusion Injury. J. Neurotrauma 26, 1369–1378.

228. Visavadiya, N.P., Patel, S.P., VanRooyen, J.L., Sullivan, P.G., and Rabchevsky, A.G. (2016). Cellular and subcellular oxidative stress parameters following severe spinal cord injury. Redox Biol. 8, 59–67.

229. Xu, W., Chi, L., Xu, R., Ke, Y., Luo, C., Cai, J., Qiu, M., Gozal, D., and Liu, R. (2005). Increased production of reactive oxygen species contributes to motor neuron death in a compression mouse model of spinal cord injury. Spinal Cord 43, 204–213.

230. Fatima, G., Sharma, V.P., Das, S.K., and Mahdi, A.A. (2015). Oxidative stress and antioxidative parameters in patients with spinal cord injury: implications in the pathogenesis of disease. Spinal Cord 53, 3–6.

231. Jia, Z., Zhu, H., Li, J., Wang, X., Misra, H., and Li, Y. (2012). Oxidative stress in spinal cord injury and antioxidant-based intervention. Spinal Cord 50, 264–274.

232. Luo, J., Li, N., Robinson, J.P., and Shi, R. (2002). The increase of reactive oxygen species and their inhibition in an isolated guinea pig spinal cord compression model. Spinal Cord 40, 656–665.

233. Lingappan, K. (2018). NF-κB in Oxidative Stress. Curr. Opin. Toxicol. 7, 81–86.

234. Maher*, P., and Schubert, D. (2000). Signaling by reactive oxygen species in the nervous system. Cell. Mol. Life Sci. CMLS 57, 1287–1305.

235. Liu, T., Zhang, L., Joo, D., and Sun, S.-C. (2017). NF-κB signaling in inflammation. Signal Transduct. Target. Ther. 2, 1–9.

236. Schreck, R., Rieber, P., and Baeuerle, P.A. (1991). Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J. 10, 2247–2258.

237. Kim, J.-H., Na, H.-J., Kim, C.-K., Kim, J.-Y., Ha, K.-S., Lee, H., Chung, H.-T., Kwon, H.J., Kwon, Y.-G., and Kim, Y.-M. (2008). The non-provitamin A carotenoid, lutein, inhibits NF-κB-dependent gene expression through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-κB-inducing kinase pathways: Role of H2O2 in NF-κB activation. Free Radic. Biol. Med. 45, 885–896.

238. Abais, J.M., Xia, M., Zhang, Y., Boini, K.M., and Li, P.-L. (2015). Redox Regulation of NLRP3 Inflammasomes: ROS as Trigger or Effector? Antioxid. Redox Signal. 22, 1111–1129.

239. Chong, W.C., Shastri, M.D., and Eri, R. (2017). Endoplasmic Reticulum Stress and Oxidative Stress: A Vicious Nexus Implicated in Bowel Disease Pathophysiology. Int. J. Mol. Sci. 18, 771.

240. Cao, S.S., and Kaufman, R.J. (2014). Endoplasmic Reticulum Stress and Oxidative Stress in Cell Fate Decision and Human Disease. Antioxid. Redox Signal. 21, 396–413.

241. Shimada, K., Crother, T.R., Karlin, J., Dagvadorj, J., Chiba, N., Chen, S., Ramanujan, V.K., Wolf, A.J., Vergnes, L., Ojcius, D.M., Rentsendorj, A., Vargas, M., Guerrero, C., Wang, Y., Fitzgerald, K.A., Underhill, D.M., Town, T., and Arditi, M. (2012). Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. Immunity 36, 401–414.

242. Szabó, C., Ischiropoulos, H., and Radi, R. (2007). Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat. Rev. Drug Discov. 6, 662–680.

243. Yoo, S.K., Starnes, T.W., Deng, Q., and Huttenlocher, A. (2011). Lyn is a redox sensor that mediates leukocyte wound attraction in vivo. Nature 480, 109–112.

244. Loo, A.E.K., Wong, Y.T., Ho, R., Wasser, M., Du, T., Ng, W.T., and Halliwell, B. (2012). Effects of Hydrogen Peroxide on Wound Healing in Mice in Relation to Oxidative Damage. PLoS ONE 7, e49215.

245. Crack, P.J., and Wong, C.H.Y. (2008). Modulation of Neuro-Inflammation and Vascular Response by Oxidative Stress Following Cerebral Ischemia-Reperfusion Injury. Curr. Med. Chem. 15, 1–14.

246. Yang, D., Elner, S.G., Bian, Z.-M., Till, G.O., Petty, H.R., and Elner, V.M. (2007). Pro-inflammatory Cytokines Increase Reactive Oxygen Species through Mitochondria and NADPH Oxidase in Cultured RPE Cells. Exp. Eye Res. 85, 462–472.

247. Pang, Q.-M., Chen, S.-Y., Xu, Q.-J., Fu, S.-P., Yang, Y.-C., Zou, W.-H., Zhang, M., Liu, J., Wan, W.-H., Peng, J.-C., and Zhang, T. (2021). Neuroinflammation and Scarring After Spinal Cord Injury: Therapeutic Roles of MSCs on Inflammation and Glial Scar. Front. Immunol. 12.

248. Lugrin, J., Rosenblatt-Velin, N., Parapanov, R., and Liaudet, L. (2014). The role of oxidative stress during inflammatory processes. Biol. Chem. 395, 203–230.

249. Nguyen, G.T., Green, E.R., and Mecsas, J. (2017). Neutrophils to the ROScue: Mechanisms of NADPH Oxidase Activation and Bacterial Resistance. Front. Cell. Infect. Microbiol. 7.

250. Kunkemoeller, B., and Kyriakides, T.R. (2017). Redox Signaling in Diabetic Wound Healing Regulates Extracellular Matrix Deposition. Antioxid. Redox Signal. 27, 823–838.

251. Krasowska, A., and Konat, G.W. (2004). Vulnerability of brain tissue to inflammatory oxidant, hypochlorous acid. Brain Res. 997, 176–184.

252. Albrich, J.M., McCarthy, C.A., and Hurst, J.K. (1981). Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leukocyte myeloperoxidase. Proc. Natl. Acad. Sci. U. S. A. 78, 210–214.

253. Wang, L., Yao, Y., He, R., Meng, Y., Li, N., Zhang, D., Xu, J., Chen, O., Cui, J., Bian, J., Zhang, Y., Chen, G., and Deng, X. (2017). Methane ameliorates spinal cord ischemia-reperfusion injury in rats: Antioxidant, anti-inflammatory and anti-apoptotic activity mediated by Nrf2 activation. Free Radic. Biol. Med. 103, 69–86.

254. Ma, Q. (2013). Role of Nrf2 in Oxidative Stress and Toxicity. Annu. Rev. Pharmacol. Toxicol. 53, 401–426.

255. Khan, M., Dhammu, T.S., Singh, I., and Singh, A.K. (2018). Amelioration of spinal cord injury in rats by blocking peroxynitrite/calpain activity. BMC Neurosci. 19, 50.

256. Numajiri, N., Takasawa, K., Nishiya, T., Tanaka, H., Ohno, K., Hayakawa, W., Asada, M., Matsuda, H., Azumi, K., Kamata, H., Nakamura, T., Hara, H., Minami, M., Lipton, S.A., and Uehara, T. (2011). On-off system for PI3-kinase-Akt signaling through S-nitrosylation of phosphatase with sequence homology to tensin (PTEN). Proc. Natl. Acad. Sci. U. S. A. 108, 10349–10354.

257. Basso, D.M., Beattie, M.S., and Bresnahan, J.C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 12, 1–21.

258. Jia, Z., Zhu, H., Li, J., Wang, X., Misra, H., and Li, Y. (2012). Oxidative stress in spinal cord injury and antioxidant-based intervention. Spinal Cord 50, 264–274.

259. Sies, H., and Jones, D.P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat. Rev. Mol. Cell Biol. 21, 363–383.

260. Casas, A.I., Nogales, C., Mucke, H.A.M., Petraina, A., Cuadrado, A., Rojo, A.I., Ghezzi, P., Jaquet, V., Augsburger, F., Dufrasne, F., Soubhye, J., Deshwal, S., Sante, M.D., Kaludercic, N., Lisa, F.D., and Schmidt, H.H.H.W. (2020). On the Clinical Pharmacology of Reactive Oxygen Species. Pharmacol. Rev. 72, 801–828.

261. Yu, D., Neeley, W.L., Pritchard, C.D., Slotkin, J.R., Woodard, E.J., Langer, R., and Teng, Y.D. (2009). Blockade of Peroxynitrite-Induced Neural Stem Cell Death in the Acutely Injured Spinal Cord by Drug-Releasing Polymer. STEM CELLS 27, 1212–1222.

CURRICULUM VITAE

[redacted]

[redacted]