Oxidative stress in spinal cord injury and antioxidant-based intervention

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Abstract:

Study design: Literature review.

Objectives: Spinal cord injury (SCI) remains a major public health issue in developed countries as well as worldwide. The pathophysiology of SCI is characterized by an initial primary injury followed by secondary deterioration. Although the etiology and pathogenesis of SCI remain to be fully understood, it has been suggested that reactive oxygen species (ROS) and oxidative stress have a significant role in the pathophysiology of SCI. Thus, alleviating oxidative stress may be an effective strategy for therapeutic intervention of SCI. The aim of this review was to describe (i) the sources of ROS as well as the major antioxidant defenses with particular attention being paid to lipid peroxidation; (ii) the biomarkers of oxidative stress in SCI and (iii) the neuroprotective effects of various compounds with antioxidative properties in animal models of SCI.

Methods: PubMed, one of the most comprehensive biomedical databases, was searched from 1976–2011. All relevant papers were read by title, abstract and full-length article.

Results: Oxidative stress is considered a hallmark of injury of SCI. Thus, alleviating oxidative stress may be an effective way of therapeutic intervention of SCI. Two of these agents, the glucocorticoid steroid methylprednisolone and the non-glucocorticoid 21-aminosteroid tirilazad, have been shown to possess significant antioxidant activities and improve recovery of SCI patients in clinical trials. Other promising botanical compounds and their molecular targets and mechanisms of action with regard to potential protection against SCI were also described. These include carotenoids and phenolic compounds.

Conclusion: ROS and oxidative stress have a significant role in the pathophysiology of SCI. Alleviating oxidative stress is be an effective strategy for therapeutic intervention of SCI. Extensive research over the past several decades has identified numerous bioactive compounds

that have antioxidative stress benefits in animal models of SCI. Thus, continued studies on bioactive compounds with ROS-scavenging capacity may lead to the development of effective antioxidant-based modalities for treating SCI in human subjects.

Keywords: spinal cord injury | oxidative stress | biomarkers | bioactive compounds

Article:

INTRODUCTION

Spinal Cord injury (SCI) is damage or trauma to the spinal cord resulting in paralysis and loss of sensation. There are B400 000 spinally injured patients in the United States with over 14 000 new injuries occurring each year.[1] SCI imposes high physical and psychological effects not only to the individual, but also to the family and the society. Depending on the location of the injury, the symptoms of SCI may include loss of movement, sensation to feel heat, cold and touch, loss of bowel or bladder control and exaggerated reflex activities as well as pain.[1]

The pathophysiology of SCI is characterized by an initial primary injury followed by a secondary phase of injury in which oxidative stress is a critical component. Primary injury results immediately from the initial trauma, which includes contusion, damage to blood vessels and axonal shearing. In contrast, secondary injury is an indirect result from primary injury initiated by trauma. It occurs in hours, days and weeks following the primary injury. Secondary injury occurs not only at the site of the initial primary injury, but also results in spreading of the lesion to adjacent, otherwise uninjured tissue. Because secondary injury to spinal cord has an important role in disease progression, it is important to understand the molecular and cellular events leading to the secondary lesion of SCI.

Although the etiology and pathogenesis of SCI remain to be further elucidated, extensive studies over the last two decades have suggested that increased formation of reactive oxygen species (ROS) and the consequent oxidative stress are important events associated with SCI. The neurons and glia in the central nervous system including spinal cord are particularly prone to oxidative and electrophilic stress due to many factors, including a high content of polyunsaturated fatty acids, a high rate of oxidative metabolic activity, intense production of reactive oxygen metabolites and relatively low antioxidant capacity.[2,3] Indeed, oxidative stress is considered a hallmark of the secondary phase of injury of SCI.[2,3] Thus, alleviating oxidative stress may be an effective way of therapeutic intervention of SCI.

METHODS

We searched MEDLINE (PubMed) and reference lists of the included articles published mainly between 1976 and 2011 using the following key words: SCI, oxidative stress, biomarkers, antioxidant, bioactive compounds and intervention.

RESULTS

ROS and oxidative stress in SCI

Reactive oxygen species (ROS). Molecular oxygen is an essential element of life, yet incomplete reduction or excitation of oxygen during aerobic metabolism generates ROS. ROS include superoxide, hydroxyl radical, singlet oxygen and hydrogen peroxide (Figure 1). Superoxide is the one-electron reduction product of molecular oxygen. If two electrons are transferred, the product is hydrogen peroxide. Transition metal ions, such as Fe2+ or Cu+ are capable of transferring a third electron to hydrogen peroxide, causing lysis of the O–O bond generating the hydroxyl radical, one of the most potent oxidants known. In phagocytes, superoxide is produced in large quantities by the enzyme nicotinamide adenine dinucleotide phosphate oxidase for use in oxygen-dependent killing mechanisms.[4] Superoxide has also been implicated in the mechanisms of aging and the peroxidation of lipids. Hydrogen peroxide is not a free radical but is nonetheless a damaging species because of its ability to penetrate biological membranes. Hydrogen peroxide is toxic to cells and can lead to further free-radical generation. For example, hydrogen peroxide reacts with reduced transition metals to form the highly reactive hydroxyl radical, which readily causes damage to DNA and other biological molecules.[5–7] Previous studies have demonstrated that the formation of ROS including hydroxyl radical, superoxide and hydrogen peroxide immediately after central nervous system injury may contribute to the pathogenesis of SCI.[8,9]

Source of ROS. Production of ROS under physiological conditions is important for normal cellular redox reactions. However, excessive generation of free radicals under pathophysiological conditions such as SCI can greatly enhance the production of ROS. Mitochondrias are the major source of cellular ROS because they consume about 90% of the oxygen utilized by cells during the oxidative phosphorylation under physiological conditions. The mitochondrial dysfunction results in further increased formation of ROS. Structural and functional alterations of the mitochondria have been found in a SCI mouse model.[10] SCI results in a cascade of secondary events including release of excitatory amino acids and disruption of calcium homeostasis, which may further cause the mitochondrial dysfunction and augmented ROS formation, leading to neuronal cell death.[2] In addition to the mitochondria, phagocytic cells such as neutrophils and macrophages also contribute to the generation of ROS following experimental SCI.4 After traumatic SCI, phagocytic cells exhibit a marked increase in oxygen consumption and generation of superoxide from membranes associated nicotinamide adenine dinucleotide phosphate oxidase. The enzyme complex transfers electrons from nicotinamide adenine dinucleotide phosphate oxidase at the cytosolic side of the membrane to molecular oxygen at the other side of the membrane resulting in the production of extracellular superoxide.[4] Other potential biological sources of ROS associated with the pathogenesis of traumatic SCI include soluble cell constituents, cytosolic oxidases, transition metals, lysosomes, peroxisomes and endoplasmic reticulum. For example, there are many cytosolic enzymes that can generate ROS via reduction of molecular oxygen in their catalytic cycles. The most notable one is xanthine oxidase, which can directly reduce molecular oxygen to superoxide and hydrogen peroxide.[11]

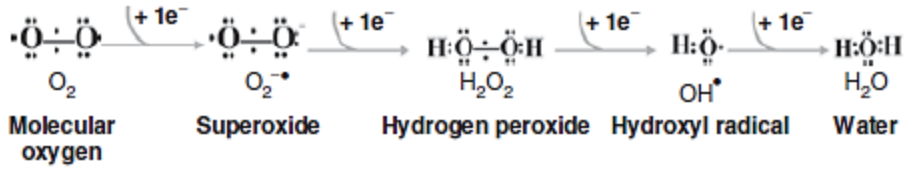

Figure 1. Schematic illustration of the formation of reactive oxygen species

Antioxidant defenses. ROS participate in physiological processes, such as cell signaling. However, excessive generation of ROS under pathophysiological conditions, including SCI may result in oxidative stress. To control ROS, aerobic organism has utilized several antioxidative mechanisms including enzymatic and non-enzymatic antioxidants[12] (Figure 2). Non-enzyme low molecular weight antioxidant compounds include cellular glutathione, vitamins C and E, β-carotene and uric acid. The antioxidant enzymes include superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidase among others. SOD catalyzes the dismutation of superoxide to H2O2 in the following reaction: O_2 + O2•− + 2H+-H2O2+O2. Mammalian cells contain three forms of SOD, MnSOD, Cu,ZnSOD and extracellular SOD. MnSOD is most abundant in the mitochondria, whereas Cn,ZnSOD predominant in the cytoplasm.[13] Catalase is a major antioxidant enzyme that catalyzes the decomposition of H2O2 to H2O. Glutathione peroxidase is another major enzyme for decomposing H_2O_2.

The elimination of ROS following SCI is particularly by cellular antioxidant glutathione and Cu,ZnSOD.14 Lucas, et al.[14] examined the effects of glutathione treatment on lipid peroxidation after SCI in a rat contusion injury model. The results suggested that elevation of levels of glutathione by irrigation with δ-glutamylcysteine conferred significant protection against lipid peroxidation after SCI, suggesting that glutathione augmentation may be an effective strategy for curtailment of lipid peroxidation-mediated damage in SCI.[14] Mutations in the gene for Cu,ZnSOD have been identified in familial amyotrophic lateral sclerosis (ALS), a disease characterized by damage and loss of neurons in both the brain and the spinal cord.[15] Transgenic mice overexpressing wide-type Cu,ZnSOD gene show resistance to SCI, indicating that Cu,ZnSOD has an important role in protecting against spinal neuron death.[16]

NAD(P)H:quinone oxidoreductase 1 (NQO1) is another important antioxidant enzyme that has recently been demonstrated to has a protective role in electrophilic stress underlying spinal cord damage.[17,18] NQO1 catalyzes the two electron reduction of electrophilic quinone compounds, thus limiting the formation of semiquinone radicals through one electron reduction, and the subsequent generation of ROS.18,19 NQO1 is also able to maintain the cellular levels of ubiquinol and vitamin E, two important biological antioxidants involved in the detoxification of ROS.[17,20] Therefore, the coordinated actions of a spectrum of cellular antioxidants and probably other cytoprotective proteins ensure efficient detoxification of ROS and electrophilic species that participate in the pathogenesis of SCI.

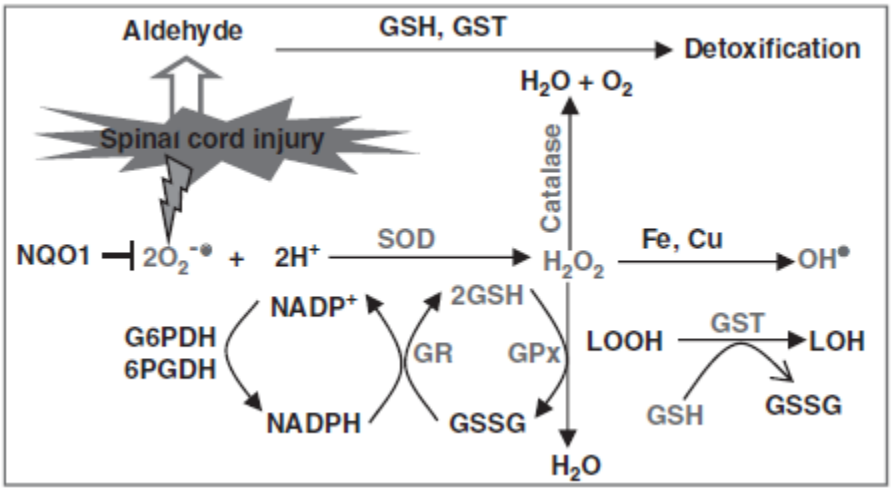

Figure 2. ROS and their detoxification by cellular antioxidants. GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized form of glutathione; GST, glutathione S-transferase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; LOH, lipid alcohol; NQO1, NAD(P)H:quinone oxidoreductase 1; SOD, superoxide dismutase.

Lipid peroxidation. High levels of ROS can overwhelm the normal cellular antioxidant defenses leading to several direct and indirect health effects. Direct effects include chain of peroxidation reactions involving lipids and other macromolecules. Indirect effects include modified metabolic pathways and altered pathophysiology of the organ systems due to the oxidative damage. Oxidative stress may occur when ROS are produced faster than they can be removed by cellular defense mechanisms. The main damage to cells results from ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, proteins, and DNA. Lipid peroxidation is a common and dangerous type of ROS-induced cellular oxidation. It has long been recognized that much of the posttraumatic degeneration of the spinal cord following injury is caused by a secondary injury process, and a significant biochemical event is ROS-induced lipid peroxidation.[2] In particular, spinal cord is highly vulnerable to oxidative injury owing to its overabundance of polyunsaturated fatty acids that are especially susceptible to peroxidation by ROS.[3] The importance of ROS and lipid peroxidation in SCI is supported by many experimental and clinical studies demonstrating potential neural protective efficacy of multiple bioactive agents with antioxidant properties.[2,21–26]

Nrf2 as a central regulator of antioxidant defenses. The coordinated elevation of antioxidant enzymes is regulated through a cis-acting element called the antioxidant response element (ARE) within the regulatory region of each of the antioxidant enzyme genes[27,28] (Figure 3). Transcriptional activation mediated by the ARE is effected by the transcription factor Nrf2. Nrf2 belongs to the CNC family of b-zip transcription factors. The mechanism by which binding of Nrf2 to ARE is induced is still emerging, but likely includes contributions from the repressor of

Nrf2 that normally resides in the cytosolic compartment along with the cysteine-rich chaperone Keap1. Keap 1 is an inhibitor of Nrf2 that sequesters it in the cytoplasm. Activation of Nrf2 dissociates Nrf2 from Keap1, allowing for its translocation to the nucleus, where it can interact with the ARE to activate transcription of antioxidant enzyme genes[27,28] (Figure 3). Activation of Nrf2 and Nrf2-mediated antioxidant enzyme gene expression by pro-oxidants, including H_2O_2 at moderate non-lethal doses suggests that Nrf2 signaling may control the adaptive response of neuronal cells to oxidative insults.[29] This potential Nrf2-mediated adaptive response to oxidative stress is particularly relevant to SCI where the increased level of ROS is constantly generated for a prolonged period of time leading to progressive oxidative stress in secondary injury of SCI.[18] Systematic increases in Nrf2 and ARE-driven heme oxygenase-1 in the spinal cord of SODG93A rats, an experimental model of ALS, have been reported,[30,31] which may represent an adaptive response to oxidative stress. In view of the critical involvement of ROS in SCI, upregulation of ARE/Nrf2 pathway to augment endogenous antioxidant defenses may serve as a unique approach to protective/therapeutic intervention of SCI.

Biomarkers of oxidative stress in SCI and their detection

Biomarkers of ROS and oxidative stress are useful for assessing the pathogenesis and progression of SCI. Biomarkers are defined as characteristics that are objectively measured and evaluated as indicators of normal biological activities, pathogenic processes or pharmacological responses to therapeutic intervention. Because the highly reactive ROS are short lived and difficult to measure directly, indirect measures are most often used to predict the amount of ROS or the extent of oxidative stress occurring in SCI. These include the measurement of glutathione, Cu,ZnSOD, malondialdehyde (MDA), acrolein and F2-isoprostanes. It is of note that each marker has its own limitation in predicting oxidation in biological systems. As such, it is recommended that at least measurement of two markers should be used.

Glutathione

Glutathione is the primary low molecular weight thiol antioxidant and co-substrate for several cellular antioxidant enzymes such as glutathione peroxidase and and glutathione transferase. It has a critical role in maintaining the intracellular oxidation–reduction (redox) balance and regulating oxidative stress-induced signaling pathways as well as detoxifying ROS and other reactive aldehydes including acrolein.[32] It is well known that the central nervous system including the spinal cord has relatively low glutathione levels compared with the other organs, such as the liver, which makes the neurons of the spinal cord especially vulnerable to oxidative stress.1,[33–35] In the central nervous system, glutathione is present at high concentrations in the astrocytes, which also provide the substrate for glutathione synthesis such as cysteine and dipeptide cysteinyl-glycine to the neighboring neurons. In addition, it is reported that glutathione may also be an important regulatory neuropeptide in the central nervous system.[36]

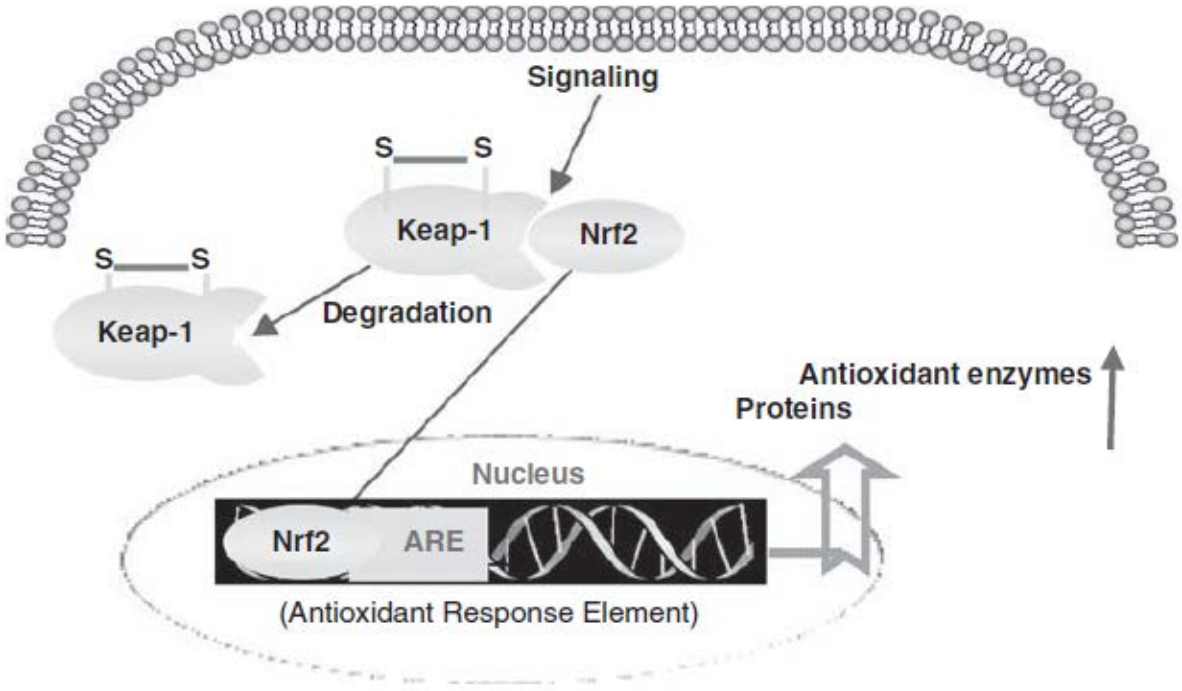

Figure 3. Nrf2 binds to the ARE and promotes transcription of antioxidant enzyme genes.

The synthesis of glutathione from its constituent amino acids involves the actions of two ATP-dependent enzymes, g-glutamylcysteine ligase (GCL) and glutathione synthase. GCL, the rate-limiting enzyme in the overall pathway, is a heterodimer composed of a catalytic (GCLC) and a modulatory (GCLM) subunit. GCLC remains all the catalytic activity and GCLM improves the catalytic efficiency. It has been demonstrated that significant decreases in glutathione occurred in spinal segments T5–T9 with the greatest decrease seen at the site of injury and immediately adjacent segments. Between 24 and 48 h, significant decreases in glutathione were also observed throughout the spinal cord from spinal segments C3 to L4, suggesting that the decrease of glutathione may be a hallmark of oxidative stress that accompanies the prominent inflammatory changes after spinal cord trauma.[37]

There are several chemical[38] and enzymatic[39] assays available for determination of glutathione. However, most of these assays lack sensitivity and specificity. Hissin and Hilf[40] developed a fluorometric method for measuring glutathione. It is based on the reaction of reduced glutathione with o-phthalaldehyde to generate a highly fluorescent adduct that is specific for determination of reduced glutathione in rat tissue at pH 8.0.[41] However, the original protocol developed by Hissin and Hilf requires a large amount of tissue samples. As such, it is not practical to routinely use the original protocol for glutathione determination in cultured neuronal cells especially under the conditions of using small volumes and microgram quantities of samples. We have modified the original method of Hissin and Hilf and reported a sensitive and specific assay for determining reduced glutathione status in human neuronal cells.[42] The modified assay allows the use of small

volumes and microgram quantities of samples, and is thus suitable for measuring reduced glutathione in a small number of cells. Given the use of a small sample volume, it also minimizes the presence of interfering substances in the samples.

Cu,ZnSOD. As mentioned before, mammalian tissues contain three forms of SOD: cytosolic Cu,ZnSOD, mitochondrial MnSOD and extracellular SOD. The presence of SOD helps dismutate superoxide immediately upon its generation and thus protects cells from superoxide-mediated oxidative damage. Several point mutations in the gene that encodes Cu,ZnSOD have been reported in some patients with familial ALS, a disease that is characterized by a loss of motor neurons in both the brain and the spinal cord.43 The cytosolic SOD activity, which is primarily Cu,ZnSOD, was reduced by 38.8% in some familial ALS patients relative to the control subjects.43,44 Furthermore, overexpression of the Cu,ZnSOD mutants in rodents emulates clinical and pathological hallmarks of ALS through a possible mechanism of toxic gain of function.[21] These results indicate a critical involvement of Cu,ZnSOD in neurodegeneration in ALS. Cellular Cu,ZnSOD activity can be measured using a commercially available kit from Trevigen, Inc. (Gaithersburg, MD, USA). This kit uses xanthine and xanthine oxidase to generate superoxide, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form formazan blue, which is monitored at 560nm. The SOD activity is then determined by measuring the %inhibition of the reaction.

Malondialdehyde (MDA). Lipid peroxidation is a well-defined mechanism of cellular damage and has been implicated in the pathogenesis of many disease processes including SCI. Lipid peroxidation is probably one of the most damaging effects of the ROS in SCI. Once a lipid radical is formed, polyunsaturated lipids can undergo self-propagating chains of peroxidation reactions. Aldehydes including MDA and the highly reactive a, b-unsaturated acrolein have been shown to be the end products of lipid peroxidation in SCI. There is increasing evidence suggesting that aldehydes may be causally involved in the pathophysiological effects associated with oxidative stress in SCI.[45]

Thiobarbituric acid-reacting substance assay is frequently used for detecting MDA. The standard or sample to be tested is heated with thiobarbituric acid at low pH, and MDA reacts with thiobarbituric acid to produce a MDA-thiobarbituric acid colored complex. Absorbance of the pink chromogen is measured at 532nm or by fluorescence at 553nm. This assay measures both free MDA and protein-bound MDA. The MDA-586 method (Oxis International, Inc., Foster City, CA, USA) is designed to assay free MDA. The assay conditions serve to minimize interference from other lipid peroxidation products, such as 4-hydroxyalkenal. The principle of the MDA-586 method is based the reaction of a chromogenic reagent, N-methyl-2-phenylindone with MDA at 45 1C. One molecule of MDA reacts with 2 molecules of N-methyl-2-phenylindone to yield a stable carbocyanine dye with maximum absorbance at 586nm.

Acrolein. Acrolein, a major byproduct of oxidative stress and lipid peroxidation, has been implicated in the pathogenesis of SCI.3,[46] As noted earlier, acrolein is a highly reactive a, b-

unsaturated aldehyde. It is produced as a byproduct of peroxidation of polyunsaturated fatty acids in cell membranes. Acrolein also occurs in the environment as a ubiquitous pollutant that is generated as a byproduct of overheated organic materials. The half-life of acrolein is estimated to be in the order of several hours, which is 100 billion times longer than that of many ROS. As such, acrolein is capable of diffusing to and injuring the otherwise healthy tissue.[3,46] Acrolein is the most potent electrophile among the unsaturated aldehydes. Acrolein-induced membrane damage may be an important pathogenic mechanism leading to cell death and functional loss in SCI.3,[46] Previous studies have demonstrated that acrolein is significantly increased following SCI, which is highly toxic to the spinal cord tissue.[3,46] As early as 4 h following SCI in vivo, acrolein was found to increase and remain elevated for at least 7 days after injury.[3,46] Notably, acrolein have been found to be elevated in the brain tissue and in the body fluids of patients with SCI.3,[46] Elevation of acrolein can deplete the cellular antioxidant glutathione as acrolein readily reacts with glutathione. This further compromises the endogenous antioxidant defenses.[3,46] Multiple studies also suggested that acrolein may represent a potential marker of oxidative stress damage to cellular constituents, especially protein targets in the spinal cord.[3,46]

The presence of protein-bound acrolein could be detected by an antibody (mAb5F6) raised against the acrolein-modified proteins using immunoblot assay.[47,48] To visualized the accumulation of acroleinmodified proteins, immunohistochemical staining can be used with the same antibody against the acrolein-modified proteins.[47,48]

8-Iso-prostaglandin F2α. 8-Iso-prostaglandin F2α is an isoprostane that has been shown to be useful for assessing oxidative stress in spinal cord ischemia.[49] It is a biologically stable end-product of the free radical-induced oxidation of arachidonic acid produced from non-cyclooxygenase and cyclooxygenase peroxidation pathways. It appears in normal plasma and urine samples and is elevated by oxidative stress. Determination of 8-iso-prostaglandin F2α has been proposed as a reliable index of oxidative stress during SCI.[50]

Several different techniques have been used to assay 8-iso-prostaglandin F2α. These include gas chromatography-mass spectrometry, enzyme immunoassay and radioimmunoassay. Recently, Basu[50] has raised an antibody specific for 8-iso-prostaglandin F2α and developed an enzyme-linked immunosorbent assay for the measurement of 8-iso-prostaglandin F2α in biological fluids. In experimental spinal cord ischemia, a significant and immediate increase of 8-iso-prostaglandin F2α in plasma at the start and up to 60min, and in the urine at 90–50 min was observed, indicating a consequence of oxidative injury.[49] Compared with other assays, the greatest advantage of this immunoassay is that the level of 8-iso-prostaglandin F2α is sufficient to be detected in every normal biological fluid, and the limit of detection is down to 23 pmol $l^{-1.}$

Antioxidant-based strategies for intervention of SCI

The involvement of ROS in the pathogenesis of SCI has prompted extensive studies on the neuroprotective effects of various compounds with antioxidative properties in animal models of

SCI. It is possible that the use of certain antioxidants may slow the progression of spinal cord damage. This section begins with a description of Cu,ZnSOD in protecting against SCI followed by summarizing the major recent findings on SCI protection by some non-protein compounds with antioxidant properties.

Cu,ZnSOD. ALS is a fatal motor neuron degenerative disease characterized by a loss of motor neurons in the central nervous system including spinal cord. Among all of the familial ALS patients, 20–30% of them have point mutations in the gene that encodes Cu,ZnSOD.43 Over 100 different missense substitutions in the 153-amino acid Cu,ZnSOD have been described in individuals and kindreds affected by Cu,ZnSOD-linked familial ALS.51 Bowling et al.44 reported that cytosolic SOD activity, which was primarily Cu,ZnSOD activity, was reduced significantly in familial ALS with SOD1 mutations. Therefore, Cu,ZnSOD is likely an important therapeutic target that protects neurons from ROS damage after SCI. Utilizing Cu,ZnSOD-overexpressing transgenic rats and a mild spinal cord compression model to induce selective death of ventral horn motor neurons, Sugawara et al.[52] reported that overexpression of Cu,ZnSOD reduced both the production of superoxide at an early stage after SCI and the subsequent ROSmediated death of ventral horn motor neurons, supporting that Cu,ZnSOD is a potential therapeutic agent. However, it is important to note the overexpression of antioxidant enzymes via genetic approaches is currently not practical for the intervention of human SCI. In this context, PEP-1, a 21-residue peptide carrier, has been developed for efficiently delivering Cu,ZnSOD fusion protein into cultured neurons and injured spinal cord in vivo.[22] Systemic administration of the fusion PEP-1-Cu,ZnSOD1 protein protected motor neurons from ROS damage and improved recovery after SCI.[22] These findings suggest that PEP-1-Cu,ZnSOD may represent a new strategy for delivery of antioxidant enzymes for therapeutic intervention of SCI.

Non-protein compounds with antioxidant properties. Vitamin E: Vitamin E is an excellent antioxidant because it is a biological compound that is naturally present in animal and human tissues. It can cross intact cell membranes and has a long half-life and good bioavailability. Pretreatment with vitamin E has been shown to be protective in animal models of SCI.[2,53] Iwasa et al. systemically studied the effects of vitamin E on injury of the spinal cord associated with ischemia in rats.[54,55] The results showed that the motor disturbance induced by SCI was greatly reduced and spinal cord blood flow was promptly restored and remained normal after injury in the vitamin E-supplemented group. Tariq et al.[56] have demonstrated that diabetic animals are more susceptible to compressive SCI as compared with nondiabetic animals most likely resulting from the higher depletion of vitamin E and glutathione in diabetic rats. Wang et al.[57] reported that vitamin E feeding attenuated some of the detrimental effects of SCI on sperm functions and male accessory glands in rats, supporting a role of ROS-related events in deterioration of SCI.

Methylprednisolone: Methylprednisolone is a synthetic glucocorticoid steroid with potent anti-inflammatory activities. Methylprednisolone has been shown to also inhibit lipid peroxidation in vitro.[2] Compression trauma of the cat spinal cord is known to induce a very rapid alteration in the lipid metabolism of cellular membranes leading to the secondary development of tissue ionic

imbalance, ischemia, edema and inflammation.[2] Studies demonstrated that pretreatment of cats with methylprednisolone (15–30mg kg^{-1}, i.v.) soon after blunt SCI decreased posttraumatic lipid peroxidation as measured by various biochemical indices.23–25 In addition to its ability to inhibit lipid peroxidation, methylprednisolone at high dose (30mg kg^{-1}, i.v.) has been shown to support energy metabolism, prevent progressive posttraumatic ischemia development, reverse intracellular calcium accumulation, ameliorate neurofilament degradation and inhibit membrane lipid hydrolysis.[2] These actions could lead to an attenuation of posttraumatic neuronal degeneration after the injured spinal cord. However, when used at high dosage for extended period of time, methylprednisolone causes serious side effects, including weight gain, glaucoma, osteoporosis and psychosis. Despite the above adverse effects, methylprednisolone treatment has been shown to be beneficial in patients with SCI.

21-Aminosteroids: 21-Aminosteroids are methylprednisolone derivatives. Compared with methylprednisolone, 21-aminosteroids lack the glucocorticoid side effects that limit the clinical usefulness of highdose methylprednisolone. The compound U-74006F is one of a series of 21-aminosteroids, which has been specifically developed for acute treatment of central nervous system trauma and ischemia due to its potent inhibition of lipid peroxidation.[2,26] U-72099E has been shown to be more potent than methylprednisolone in protecting rat brain synaptosomes from lipid peroxidation-induced inhibition of 14C-GABA uptake.[2,26] In addition, it equaled the ability of methylprednisolone to enhance the early neurological recovery of head injured mice.[2] The dose-response characteristics and capability of U-74006F to promote functional recovery in cats subjected to compression trauma of the upper lumbar (L-2) spinal cord have also been evaluated.[58] Results showed that over a 100-fold range of doses, U-74006F has a remarkable capacity to promote functional recovery in spinal cord injured cats. The mechanism of protective action of U-74006F against SCI is believed to involve an inhibition of oxygen radical-mediated lipid peroxidation. In vivo studies have demonstrated that U-74006F can preserve tissue vitamin E levels in central nervous system trauma.[59] In a manner similar to vitamin E, U-74006F has been found to scavenge lipid peroxyl radical thereby inhibiting lipid radical chain reactions.26 U-74006F also decreased hydroxyl radical formation from Fenton reactions like due to its ability to chelate redox-active iron.[2]

3H-1,2-dithiole-3-thione: as noted earlier, the central nervous system is particularly vulnerable to oxidative stress due to many intrinsic factors. Notably, the central nervous system also has relatively low glutathione levels compared with the other organs such as the liver. Because the coordinated actions of glutathione and a spectrum of other cellular antioxidative enzymes are essential for efficient detoxification of ROS that participate in the pathogenesis of SCI, we propose a novel strategy for protective/therapeutic intervention of SCI through coordinated upregulation of endogenous antioxidants in the neuronal cells mediated by 3H-1,2-dithiole-3-thione (D3T). D3T is a constituent of cruciferous vegetables. We have recently reported that D3T increases multiple cellular antioxidants including glutathione and NQO1, two crucial cellular defenses against oxidative and electrophilic stress in human neuroblastoma cells (SH-

SY5Y), human primary neurons and astrocytes, suggesting that D3Tmediated antioxidant induction is not cell-type specific.[60–62] In addition, D3T treatment of the neuronal cells also results in a marked elevation of glutathione content in the mitochondrial compartment,[62] a critical intracellular target for oxidative and electrophilic stress.[63] It is important to note that the D3T upregulated endogenous defenses are accompanied by increased cellular resistance to neurocytotoxicity elicited by ROS and several well-known neurotoxins including acrolein, suggesting a potential therapeutic value in SCI.[60–62]

Table 1. Other major agents shown to be protective against SCI in experimental models

Agents	Experimental models	Results /mechanisms of action	Reference
Scutellaria baicalensis (EESB), a herbal remedies in oriental Medicine	Primary microglial cultures and rat model following a spinal cord contusion injury	EESB treatment significantly improved functional recovery by inhibiting inflammation and oxidative stress after injury both in vivo and in vitro.	66
A novel peptide antioxidant (SS-31)	In vitro and in the G93A mouse model of amyotrophic lateral sclerosis (ALS)	Administration of SS-31 in G93A ALS mice before the onset of symptoms led to a significant increase in survival and improvement of motor performance. SS-31-treated mice showed a decreased cell loss and a decrease in immunostaining for markers of oxidative stress in the lumbar spinal cord.	67
Polyethylene glycol (PEG), a hydrophilic polymer	Guinea pig SCI model	PEG treatment decreased ROS elevation and lipid peroxidation induced by SCI, possible through inhibiting superoxide mechanism.	68
Edaravone (3-methyl-1-phenyl-pyrazolin-5-one), a newly developed radical scavenging agent	Murine model of incomplete SCI	Pretreatment with edaravone significantly improved motor dysfunction and ameliorated tissue damage by scavenging ROS, especially in the neurons, after SCI.	69
2,4-Dinitrophenol (DNP), a mitochondrial uncoupling agent	Rat model following a mild to moderate spinal cord contusion injury	Pretreatment with DNP maintained mitochondrial bioenergetics and significantly decreased ROS levels, lipid peroxidation, and protein carbonyl content following SCI.	70
Hypericum perforatum, a medicinal plant species containing many polyphenolic compounds	An experimental mouse model of SCI	Hypericum perforatum (30mg kg^{-1} bw) has strong anti-inflammatory properties resulting in a reduced histological damage, polymorphonuclear leukocyte infiltration, formation of PAR and nitrotyrosine, STAT-3 activation, NF-kB activation, and degree of apoptosis.	71
H-290/51, an antioxidant compound	A rat model of SCI	Treatment with H-290/51 (50mgkg-1, p.o.) 10 and 30min after SCI markedly attenuated c-fos expression and motor dysfunction suggesting that this antioxidant is capable of attenuating cellular and molecular events following trauma.	72
Alpha-Phenyl-N-tert-butyl Nitrone (PBN), a free radical scavenger	A rat model of severe SCI	PBN pretreatment attenuates lactic acidosis and improves energy metabolism after severe SCI suggesting the amelioration of radical induced mitochondrial dysfunction.	73
Tetramethylpyrazine	In a rat model of spinal	Treatment with TMP exerted a	74

(TMP)	cord ischemiareperfusion injury	neuroprotective effect against spinal cord ischemia-reperfusion injury.	
Resveratrol, a natural phenolic compound	A rat model of SCI	Resveratrol significantly promotes the recovery of rat dorsal neuronal function after SCI, and this effect is related to its characteristics of antioxidation, anti-inflammation and anti-apoptosis.	75
Resveratrol	A rabbit model of SCI	Prophylactic use of resveratrol reduced neurologic injury and provided clinical improvement by attenuating the inflammatory milieu in the rabbit spinal cord ischemia/reperfusion model.	76
U74389F, a compound in a family of 21-aminosteroids that inhibit lipid peroxidation	A rat model of SCI	The bolus administration of U74389F one hour after injury facilitates the return of the spinal cord function as measured by the CSEPs in this compression model of acute spinal cord trauma.	77
Neu2000, a novel NMDA antagonist and antioxidant	A rat model of moderate SCI	Neu2000 treatment significantly reduced the production of mitochondrialfree radicals and improved locomotor outcomes that were associated with a significant increase in the volume of spared spinal cord tissue.	78
Quercetin, a flavonol	A rat model of SCI	Quercetin administration results in preservation of tissue bridges at the site of injury. Treatment success depends on frequency of administration and overall dose.	79
Curcumin, a member of the ginger family (Zingiberaceae)	A rat model of SCI	Curcumin inhibited apoptosis and neuron loss, quenched astrocyte activation, and significantly improved neurologic deficit 7 days after spinal cord hemisection.	80
Caffeic acid phenethyl ester (CAPE), a component of propolis	A rat model of SCI	CAPE enhanced the recovery of locomotor function and reduced the lesion size while suppressing the expression of the mRNAs for a pro-inflammatory cytokine.	81
Epigallocatechin Gallate (EGCG)	A rat model of SCI	MDA levels were significantly decreased in EGCG treatment groups. EGCG significantly reduced the percentage of lesion area and improved behavioral function than the trauma group.	82
NAC (N-acetyl-cysteine), a precursor to glutathione	A rabbit mode of spinal cord ischemia-reperfusion (I-R)	NAC showed protective effects of the spinal cord.	83
Baicalin (BC), a flavone	A rat model of SCI	The BC therapy ($100 mg kg^{-1}$) dramatically decreased the water content of spinal cord tissue, the permeability of blood-spinal cord barrier, oxidant stress and proinflammatory cytokines expression. Also, the treatment with BC also significantly improved the recovery of limb function.	84
Polyethylene glycol (PEG)	A rat model of SCI	Post-SCI administration of PEG decreased lesion volume, increased neurofilament-positive fibers and corticospinal tract fibers in the lesion, and did not increase reactive gliosis.	85

Taurine, a potent antioxidant	A rat model of SCI	Taurine significantly decreased IL-6 and MPO levels in a dose-dependent manner and significantly reduced the phosphorylation of STAT3 and expression of COX-2 after SCI compared to controls.	86
Alpha-tocopherol	A rat model of SCI	The administration of alpha-tocopherol significantly improved the mean motor score compared with control group due to its antioxidant effect.	87
Alpha-tocopherol	In a rat model of spinal cord ischemia-reperfusion Injury	Alpha-tocopherol administration improves the oxidative stress level and significantly prevents the damage caused by spinal cord ischemia-reperfusion injury with subsequent recovery of both motor and sensory functions.	88
Methylprednisolone (MP) and the nonglucocorticoid 21-aminosteroid tirilazad	Acute SCI	Administration of these compounds has been demonstrated in the multicenterNASCIS clinical trials to produce at least a modest improvement inneurological recovery when administered within the first 8 hours after SCI.	89
Curcumin	A rat model of SCI	Curcumin administration increases SOD level and significantly prevents the damage (decreased MDA level) compared to control group indicating that curcumin effectively protects the spinal cord tissues against oxidative damage.	90
Lipoid acid (LA)	In a rat model of spinal cord ischemia-reperfusion injury	LA pretreatment reduced neurologic injury in the rats, most probably by maintaining the oxidant/anti-oxidant ion balance during spinal cord ischemia.	91
High doses of vitamins C and E (100mg kg^{-1} day^{-1})	A rat model of SCI	The use of vitamins C and E did not improve their neurological performance. However, histopathological examination showed that the inflammatory response was less intense following administration of the combination of vitamins C and E.	92
Aluminum	A rat model of SCI	Administration of aluminum significantly impaired the recovery following SCI. Analysis of the results of the biochemical, electrophysiological, and histopathological studies also confirmed the deleterious effects of aluminum on recovery from SCI in rats.	93
17beta-estradiol	In a rat model of SCI	17beta-estradiol protects schwann cell (SCs) against oxidative stress and improves transplanted SC survival.	94
Tadalafi	In a rat model of SCI	Tadalafil is beneficial in reducing the effects of injury to the spinal cord by increasing tissue levels of NO and serum activity of SOD.	95
Acetyl-L-carnitine (ALC)	In a rat model of SCI	ALC treatment maintains mitochondrial bioenergetics following contusionSCI.	96
Sulforaphane	A murine model of SCI	Suforaphane decreases MMP-9, TNF-alpha expression and vascular permeability changes following SCI in mice.	97

Deferoxamine	In a rat model of SCI	Deferoxamine reduced the levels of free iron and lipid peroxidation, and improved the hind limb functional status of rats with SCI.	98
Epigallocatechin gallate (EGCG)	In a rat model of SCI	EGCG is effective in protecting rat spinal cord from secondary injury by reducing the percentage of lesion area and improving behavioral function.	99
Infliximab	A rabbit I/R model	Infliximab protected the spinal cord against injury in a rabbit I/R model with decreased level of MDA in the tissue.	100
Baicalin (BC)	In a rat model of SCI	BC treatment significantly decreased oxidative stress and attenuated the SCI.	
Ethyl pyruvate (EP)	A rabbit model of SCI	EP affords a strong protection against the transient spinal cord ischemic injury through inhibition of HMGB1 release.	101
Dantrolene	A rabbit model of SCI	Dantrolene DNT treatment prevented lipid peroxidation by SCI, and augmented endogenous enzymic or non-enzymic antioxidative defense systems.	102
Melatonin	A rabbit model of SCI	Melatonin administration reduced the incidence of SCI with decreased MDA levels and increased glutathione in serum and tissue.	103
Octreotide and melatonin	In a rat model of SCI	Melatonin was found to be superior to octreotide to attenuate the SCI.	104
Lecithinized superoxide dismutase	In a rat model of SCI	Lecithinized superoxide dismutase suppressed neuronal death through reducing oxidative stress.	105
Polyunsaturated fatty acids.	In a rat model of SCI	Omega-3 PUF was found to be neuroprotective and omega-6 PUFAs had a damaging effect after SCI in the adult rat.	106
Nigella sativa (NS)	In a rat model of SCI	NS treatment is beneficial in spinal cord tissue damage through reducing oxidative stress.	107
MnTBAP	In a rat model of SCI	Treatments with the low doses of MnTBAP provide sustained neuroprotection by preventing oxidative stress.	108
Ginkgo leaf extracts	In a rat model of SCI	Ginkgo leaf extracts afford a strong protection against SCI through reducing lipid peroxidation injury and inhibiting apoptosis.	109
Resveratrol and methylprednisolone (MP)	In a rat model of SCI	Resveratrol treatment revealed better biochemical recovery in the acute stage of trauma than MP treatment and combinations promise better results as each drug has a different anti-oxidative mechanism of action.	110
Ebselen	In a rat model of SCI	Ebselen treatment decreased tissue MDA level and prevented inhibition of the enzymes SOD, GSH-Px and CAT in the tissues induced by SCI.	111
Glutathione monoethyl ester (GSHE)	In a rat model of SCI	GSHE treatment improved functional outcome and red nuclei neuron survival of SCI.	112
Polyethylene glycol (PEG).	In a guinea pig model of SCI	PEG exerted its neuroprotective effect through direct interaction with mitochondria	113

		and reducing oxidative stress.	
Resveratrol	A rabbit I/R model	Resveratrol-induced neuroprotection is mediated by its antioxidant and NO promoting properties.	114
Melatonin, and oxytetracycline	In a rat model of SCI	Melatonin and oxytetracycline are effective in preventing lipid peroxidation in SCI.	115
H-290/51	In a rat model of SCI	The antioxidant compound H-290151markedly attenuated the traumainduced oxidative stress.	116
Glutathione	In a rat model of SCI	Glutathione significantly attenuated LPO-mediated damage in acute phase SCI.	117
BDNF	In a rat model of SCI	Infusion of BDNF inhibited the acute down-regulation of CuZnSOD expression.	118
Methylprednisolone (MP), tirilazad mesylate (TM) and vitamin E	In a rat model of SCI	Treatments of MP, TM and vitamin E afforded a protective effect against SCI in rats by their antioxidant effects.	119
Methylprednisolone sodium succinate (MPSS).	In a rat model of SCI	MPSS administration significantly decreased the level of lipid peroxidation and protected spinal cord ultrastructure following SCI.	120
Caffeic acid phenethyl ester (CAPE)	A rabbit I/R model	CAPE protected the spinal cord from ischemia-reperfusion injury through its antioxidant effect.	121
Alpha-tocopherol	In a rat model of SCI	Long term administration of alpha-tocopherol decreased lipid peroxidation following acute spinal cord trauma.	122

Other compounds: several epidemiological studies have demonstrated that increased consumption of antioxidant-rich fruits and vegetables is associated with reduced risk of ischemic stroke, dementia and SCI.[64,65] In this context, identification and study of the biologically active compounds may be of importance for developing strategies to inhibit, retard or reverse the pathophysiological processes underlying SCI. Beside the aforementioned agents with antioxidant properties for treating SCI, there are numerous dietary and botanical natural compounds in fruits, vegetable and plants (such as green tea compounds and herbal remedies) being actively investigated for their potential benefits in SCI. These include carotenoids and phenolic compounds. Carotenoids are ubiquitous in the plant kingdom, and as many as 1000 naturally occurring variants have been identified. Plant phenols are antioxidants by virtue of the hydrogen donating properties of the phenolic hydroxyl groups, and over 8000 plant phenols have been isolated. Dietary plants rich in these compounds include broccoli, brussel sprouts, cabbage, kale, cauliflower, carrots, onions, tomatoes, spinach, garlic and some herbal medicines. Additionally, some compounds found in these plants may improve the endogenous antioxidant defense through induction of antioxidant enzymes.

Table 1 lists some bioactive compounds derived from different sources showing benefits in protecting against oxidative stress underlying SCI. These compounds have been shown to suppress lipid peroxidation due to their antioxidant properties including scavenging ROS, chelating redox-active metal ions and inhibiting xanthine oxidase. However, the exact cell signaling pathways activated by these natural bioactive agents are still not clear, and each

compound may act differently in different cells. Studies have suggested that the protective role of these compounds against oxidative stress damage is mediated, at least partially, by effects on signaling molecules including extracellular signal-regulated kinase, nuclear factor kB and Nrf2.1[8,27–31] Nrf2 signaling appears to has a central role in regulating the induction of antioxidants by phenolic compounds.[18,27–31]

CONCLUSION

The central nervous system, including spinal cord is highly susceptible to free-radical-mediated damage due to its high lipid content and active oxygen metabolism. It has been demonstrated that ROS and oxidative stress have a significant role in the pathophysiology of SCI and are a hallmark of the secondary injury underlying SCI in animal models. Thus, alleviating oxidative stress of secondary injury process may represent an effective strategy for therapeutic intervention of SCI. In this regard, continued studies on bioactive compounds with ROSscavenging capacity may lead to the development of effective antioxidant- based modalities for treating SCI in human subjects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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