ORIGINAL ARTICLE

Reduced Antioxidant Defense and Increased Oxidative Stress in Spinal Cord Injured Patients

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ABSTRACT. Bastani NE, Kostovski E, Sakhi AK, Karlsen A, Carlsen MH, Hjeltnes N, Blomhoff R, Iversen PO. Reduced antioxidant defense and increased oxidative stress in spinal cord injured patients. Arch Phys Med Rehabil 2012;93:2223-8.

Objective: To determine the plasma and urine levels of antioxidants and oxidative stress biomarkers in subjects with spinal cord injury (SCI) the first year after injury.

Design: Descriptive 1-year follow-up study.

Setting: Rehabilitation and research center.

Participants: SCI subjects (n=37; age range, 18–70y) consecutively enrolled within the first month after injury. A healthy, able-bodied control group (n=346) was also included.

Interventions: Not applicable.

Main Outcome Measures: Blood and urine levels of antioxidants and oxidative stress biomarkers were measured at inclusion and after 3 and 12 months postinjury.

Results: One month after injury, the plasma antioxidants (total and oxidized glutathione and 6 different carotenoids and α-tocopherol) were reduced by 19% to 71% among the SCI subjects compared with the controls. The redox potential was reduced by 7% among the SCI subjects. The oxidative stress biomarker urinary 8-epi prostagladin F2α (PGF2α) increased to 161% in the SCI subjects compared with the controls. After 3 and 12 months, most of the antioxidant biomarkers were still significantly reduced compared with the controls, while urinary 8-epi PGF2α had increased to 208% compared with the controls.

Conclusions: The levels of antioxidants were significantly lower, while the marker of oxidative stress was higher in the SCI subjects compared with the controls. This observation demonstrates that SCI patients experience increased oxidative stress and reduced antioxidant defense the first year after injury. Our findings warrant intervention studies where SCI patients receive dietary antioxidant support as part of their rehabilitation.

Key Words: Antioxidants; Oxidative stress; Quadriplegia; Rehabilitation.

IN THE NORDIC COUNTRIES, between 10 and 20 persons per million inhabitants annually suffer a traumatic spinal cord injury (SCI) that causes permanent and severe disability.[1-5] Patients with injury to 1 of the 8 cervical segments or the first thoracic segment of the spinal cord have tetraplegia, whereas those with paraplegia have lesions in 1 of the remaining caudal segments. Persons with incomplete lesions have spared sensory and/or motor function in the sacral segments and varying degrees of muscle function below the injury level.[6] Among those with incomplete SCI, motor and sensory function recovery occurs mostly within the first year after injury (early phase), although some also may have improvement in functions later (chronic phase).[7] The most frequent neurologic deficits after traumatic SCI are incomplete tetraplegia, followed by complete paraplegia, incomplete paraplegia, and complete tetraplegia.[8]

An SCI is characterized by primary damage that is defined as the immediate effects of an injury to the spinal cord, most commonly caused by disruption, contusion, or compression. Then, secondary damage occurs in the following hours or days, which is caused by the body's local and general response to the trauma.[9,10] Although the pathophysiologic mechanisms underlying the primary and secondary damages in SCI are incompletely understood, oxidative stress is believed to be involved.[11] The generation of reactive oxygen species (ROS) (eg, free radicals) is a normative response to diseases or injuries. However, increased formation of the ROS can exceed the capacity of the antioxidant defense and may thus mediate oxidative damage and subsequently oxidative stress.[12,13]

A number of endogenous and exogenous antioxidants are important for the antioxidant defense. The tripeptide glutathione (GSH) is an essential endogenous small molecular weight thiol present in plasma and in all cells in the body.[14]

The oxidation-reduction (redox) of plasma GSH, that is, the redox state of the GSH/oxidized glutathione (GSSG) couple, was estimated using the Nernst equation, as described by

List of Abbreviations

AIS	American Spinal Injury Association Impairment Scale
Cys	cysteine
Cys/Gly	cysteinyl-glycine
GSH	glutathione
GSSG	oxidized glutathione
Hcy	homocysteine
HPLC	high-pressure liquid chromatography
PGF2α	prostagladin F2α
ROS	reactive oxygen species
SCI	spinal cord injury

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Schafer and Buettner.[15] A more negative value of GSH redox potential indicates more reduced GSH/GSSG redox state, and hence higher reducing capacity of GSH. An increase in GSSG in plasma decreases the GSH/GSSG ratio and results in more positive GSH redox potential, and hence lowers reducing capacity. In addition to the free reduced and oxidized forms of GSH in plasma, GSH is also bound to other thiol compounds (eg, cysteine) and proteins.[16] Therefore, the total GSH is also a measure of oxidative stress.

Carotenoids are lipid soluble plant pigments with antioxidant activity. Among the estimated 750 naturally known carotenoids, lutein, zeaxanthin, β-kryptoxanthin, α-carotene, β-carotene, and lycopene are the most studied.[17] Plasma carotenoids may also serve as biomarkers for fruit and vegetable intake.[18,19] Vitamin E (α-, β-, γ-, and δ-tocopherols) is considered to be the major lipid-soluble antioxidant to protect cell membrane from oxidative damage.[20]

Isoprostanes are biologically active compounds formed by oxidation of polyunsaturated fatty acids. Urine isoprostane 8-epi prostagladin F2α (PGF2α) is often used as a biomarker for oxidative stress.[21]

Despite the postulated role of oxidative stress in the development and progression of SCI, there is only limited information about oxidative stress and the status of the antioxidant system in SCI patients.[11] Additionally, very little is known about temporal changes in oxidative stress after an acute trauma to the spinal cord. Moreover, the possible impact of oxidative stress on the severity of SCI remains largely unknown.

In this study we have used a broad panel of antioxidant and oxidative stress biomarkers to determine antioxidant status in SCI patients 1, 3, and 12 months after acute trauma to the spinal cord in otherwise healthy subjects and compared the results with those from able-bodied controls.

METHODS

Participants and Study Design

We invited 85 adult (range, 18–70y) men and women with acute SCI hospitalized in Sunnaas Hospital (Nesoddtangen, Norway) from January 2007 to July 2009 to participate in the study. While 48 SCI subjects declined the invitation or did not fulfill the inclusion criteria, 37 otherwise healthy SCI subjects were included in the study. In particular, they did not suffer from any immunologic, inflammatory, or malignant disease, and they did not use any drugs regularly except for infrequent use of spasmolytics. Blood and urine samples were collected from the SCI subjects within the first month after injury, at discharge from Sunnaas Hospital (ie, after about 3mo), and as outpatients 1 year or more (chronic phase) after the trauma. Because of transfer to other hospitals or discharge, we were not able to obtain blood and urine samples from all patients. Samples were obtained from 37, 26, and 33 SCI patients after 1, 3, and 12 months, respectively.

An SCI is classified by the American Spinal Injury Association Impairment Scale (AIS), which indicates the level of remaining sensory and/or motor function below the spinal level of injury. In short, AIS grade A injury indicates no preserved motor or sensory function, AIS grade B indicates preserved sensory function, but no preserved motor function, and AIS grades C and D injuries indicate preserved sensory function with varying degrees of loss of motor function.

Additionally, we used data obtained from a control group consisting of 346 healthy individuals originally included in another study conducted at the University of Oslo.[22,23] These 346 participants were recruited after response to an invitation letter sent to a random selection of adult citizens in Oslo and its surrounding area.

The present study was conducted according to the Declaration of Helsinki guidelines. All procedures were approved by the Regional Committee for Medical and Health Research Ethics South, and all participants gave written informed consent at the time of inclusion.

Blood and Urine Sampling

Venous blood and spot urine were sampled from SCI patients in the morning after an overnight fast. Plasma and urine aliquots were snap-frozen in liquid nitrogen and stored at −80°C. Overnight, fasting plasma samples were available from all 346 control subjects, whereas 24-hour urine samples were available from 132 control subjects.

Measurement of Plasma GSH/GSSG

The determination of GSH and GSSG by high-pressure liquid chromatography (HPLC) has been detailed by Sakhi et al.[24] The plasma obtained was prepared for analyses of reduced and oxidized forms of GSH, as described in Sakhi et al.[25] The redox potential ratio between GSH and GSSG was calculated with the Nernst equation as described by Schafer and Buettner[15]:

$$GSSG + 2H^+ + 2e^- \rightarrow 2GSH$$

$$E_{hc} = -240 - (59.1/2) * \log([GSH]^2/[GSSG])\ mV$$

Here, E_{hc} is the half-cell reduction potential. The standard potential E_0 for the 2GSH/GSSG couple was −264 mV, based on the value of −240 mV for pH 7.0, and a pH effect of −59 mV/pH unit.

Measurement of Plasma Thiols

The levels of total GSH, cysteine (Cys), homocysteine (Hcy), and cysteinyl-glycine were analyzed using the Homocysteine by HPLC kit.[a] Calibration, sample preparation, and HPLC were performed as described elsewhere.[26]

Measurement of Urinary F2-Isoprostane and Creatinine

The determination of the isoprostane 8-epi-PGF2α was performed by liquid chromatography–mass spectrometry, as described by Bastani et al.[27]

The creatinine level in urine used for the normalization of 8-epi-PGF2α concentration (8-epi-PGF2α/mg creatinine) was measured at the Department of Medical Biochemistry, Oslo University Hospital, Ullevål.

Measurement of Plasma Carotenoids

For analysis of carotenoids (lutein, zeaxanthin, β-kryptoxanthin, α-carotene, β-carotene, and lycopene) in plasma, HPLC with ultraviolet detection was used. Proteins in plasma samples were precipitated by the addition of a 4.5 times volume of isopropanol containing 5μg/mL astaxanthin (internal standard). Samples were mixed for 10 minutes and then centrifuged (3000×*g* at 4°C) for 15 minutes. Aliquots of 20μL of the supernatant were injected into the HPLC system. The mobile phases consisted of A (20% water and 24% acetone in ethanol) and B (100% acetone). The separation was achieved with a linear gradient from 2% to 100% mobile phase B within 20 minutes, followed by 100% mobile phase B for 15 minutes. The flow rate was 0.3mL/min. Detection was performed at 453nm. Plasma calibrators and controls were quantified against the standardized reference material 968c from the National Institute of Standards and Technology.