Simultaneous Activation of Nrf2 and Elevation of Antioxidant Chemicals in Management of Post-Traumatic Stress Disorders and Traumatic Brain Injury

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Abstract

Despite animal and human studies and improved understanding of the symptoms and cellular damage, current managements of post-traumatic stress disorders (PTSDs) and traumatic brain injury (mild TBI and penetrating TBI) remain unsatisfactory. Despite differences in etiology and symptoms, three common biochemical defects increased oxidative stress; chronic inflammation and glutamate release that contribute to the initiation and progression of these diseases were identified. Therefore, attenuation of these biochemical abnormalities may reduce the progression, and in combination with standard care, may improve the management of these neurological disorders. This review proposes that an elevation of the levels of antioxidant enzymes and phase-2-detoxifying enzymes, and dietary and endogenous antioxidants simultaneously is essential for optimally attenuating oxidative stress, chronic inflammation, and glutamate release. The levels of antioxidants are increased by supplementation; however, an activation of a nuclear transcriptional factor Nrf2 and its binding with antioxidant response elements (AREs) is required for increasing the levels of antioxidant enzymes and phase-2-detoxifying enzymes. This review briefly discusses the regulation of Nrf2 activation, and identifies agents that activate Nrf2 by reactive oxygen species (ROS)-dependent and -independent mechanisms. Studies on the effects of antioxidants in PTSD and TBI are presented to show that the use of a single agent cannot activate Nrf2 and enhance the levels of dietary and endogenous antioxidants simultaneously. The proposed micronutrient cocktail can achieve the above goal, and thereby, may reduce the progression, and in combination with standard care, improve the management of PTSD and TBI.

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a complex mental disorder with psychological and emotional components. It is caused by the exposure to single or repeated extreme traumatic events found in war, terrorist attacks, natural or man-caused disasters, and by violent personal assaults and accidents. Mild traumatic brain injury (mildTBI) occurs when the brain is violently rocked back and forth within the skull following a blow to the head or neck as in contact sports, such as football, Soccer, ice hockey and boxing or when in close proximity to a blast pressure wave following detonation of explosives in the battlefield. Penetrating TBI occurs when an object penetrates the skull and damages the brain causing bleeding and loss of varying amounts of tissue. It is caused by vehicle crashes, gunshot wound to the head, and exposure to solid fragments in the proximity of explosions, and other combat-related head injuries. Because of current military conflicts around the world, the incidence of PTSD and TBI among the troops and civilian will continue to increase.

In order to reduce the impact of risk factors of PTSD and TBI on brain function, current efforts have focused on the development of physical protection of outer skull by helmets/headgears. Despite state-of-the-art physical protection, increased number of US troops continues to suffer from PTSD and TBI. This may be due to the fact that blast pressure wave following detonation of an improvised explosive device (IED)
can penetrate helmets/headgears causing adverse physiological and biochemical changes in the brain. Currently available physical protective devices may protect the skull from the direct impact of the risk factors, but they may not be sufficient to decrease the progression of injury leading to dementia and abnormal behavior. At present, treatments of PTSD and mild TBI involving medications, physical therapy and psychotherapy are not considered satisfactory.

Despite surgical intervention, medications and hypothermia; treatment strategies for patients with penetrating TBI remain unsatisfactory, and morbidity and mortality continue to be high. In addition, the risk of dementia and other forms of mental disorders among survivors of penetrating TBI continue to occur despite modern treatments.

To develop an improved treatment plan for reducing the progression and improved management of PTSD and TBI, it is important to identify underlying biochemical defects that play a key role in the initiation and progression of these disorders. Analysis of previous published studies led to the identification of three common biochemical defects that include increased oxidative stress, chronic inflammation and glutamate release. These biochemical abnormalities contribute to the development and progression of PTSD, mild TBI and penetrating TBI [1]. How to reduce these biochemical abnormalities simultaneously remain a challenging problem for basic researchers and clinicians.

An activation of a nuclear transcriptional factor Nrf2 (nuclear factor-erythroid 2-related factor-2), which increases the expression of target genes coding for antioxidant enzymes and phase-2-detoxifying enzymes, can be used as a target for developing new therapeutic drugs for chronic neurodegenerative diseases. However, activation of Nrf2 alone may not be sufficient to optimally decrease the levels of oxidative stress, chronic inflammation and glutamate release, because dietary and endogenous antioxidants also decrease in the presence of a high oxidative environment; therefore, their levels must also be elevated at the same time. Antioxidants are known to reduce oxidative stress by scavenging free radicals, but their role in reducing inflammation [2-9], and glutamate release [10] and glutatione-induced neurodegeneration and death [11, 12], are not widely appreciated. The role of B-vitamins in reducing the release of glutamate [13-15] is not widely recognized.

The antioxidant enzymes reduce oxidative stress in part by a mechanism that is different from that of antioxidant chemicals. Antioxidant enzymes destroy free radicals by catalysis, whereas antioxidant chemicals decrease by directly scavenging them. On the other hand, phase-2-detoxifying enzymes removed damaged molecules from the cells. Therefore, the levels of both antioxidant enzymes, phase-2-detoxifying enzymes, and dietary and endogenous antioxidants must be elevated simultaneously to optimally reduce oxidative stress, chronic inflammation and glutamate release. The levels of multiple dietary and endogenous antioxidants can be increased by supplementation; however, increasing the levels of antioxidant enzymes and phase-2-detoxifying enzymes is complex requiring an activation of Nrf2. Normally, in response to increased reactive oxygen species (ROS), Nrf2 is activated and translocated from the cytoplasm to the nucleus where it binds with the AREs which then increased the expression of target genes coding for antioxidant enzymes and phase-2-detoxifying enzymes [16, 17]. During chronic oxidative stress found in PTSD and TBI, Nrf2 becomes resistant to ROS. This is evidenced by the fact that increased oxidative stress is found in patients with PTSD and TBI despite the presence of Nrf2. The questions arise as to how to activate ROS-resistant Nrf2 and how to elevate multiple dietary and endogenous antioxidants simultaneously. Supplementation with a single antioxidant commonly used in most clinical studies on chronic neurodegenerative diseases cannot activate Nrf2 and elevate multiple antioxidant chemicals simultaneously.

This review propose a hypothesis that an elevation of the levels of antioxidant enzymes and phase-2-detoxifying enzymes, and multiple dietary and endogenous antioxidant chemicals simultaneously may be necessary for optimally reducing oxidative stress, chronic inflammation and glutamate release. The regulation of activation of Nrf2, which is necessary for increasing the levels of antioxidant enzymes and phase-2-detoxifying enzymes, is briefly reviewed, and antioxidant chemicals that can activate Nrf2 by ROS-dependent and -independent mechanisms are identified. Laboratory and human studies with the individual micronutrients on the progression and improved management of PTSD and TBI are briefly described in order to emphasize that supplementation with a single micronutrient cannot activate Nrf2 and enhance the levels of multiple dietary and endogenous antioxidants simultaneously. This review proposes a micronutrient cocktail that can achieve the above goal, and thereby, may reduce the progression, and in combination with standard care, improve the management of PTSD and TBI

Regulation of Nrf2 Activation

Regulation of Nrf2 activation by a ROS-dependent mechanism: The nuclear transcriptional factor, Nrf2 (nuclear factor-erythroid-2-related factor 2) belongs to the Cap ‘N’Collar (CNC) family that contains a conserved basic leucine zipper (bZIP) transcriptional
factor [18]. Normally, Nrf2 is associated with Kelch-like ECH associated protein 1 (Keap1) which acts as an inhibitor of Nrf2 [19]. Keap1 protein serves as an adaptor to link Nrf2 to the ubiquitin ligase Cul-Rbx1 complex for degradation by proteasomes and maintains the steady levels of Nrf2 in the cytoplasm. Nrf2-keap1 complex is primarily located in the cytoplasm, Keap1 acts as a sensor for ROS/electrophilic stress. In response to increased ROS, Nrf2 is activated and then dissociates itself from Keap1- Cul-Rbx1 complex and translocates in the nucleus where it heterodimerizes with a small Maf protein, and binds with ARE leading to increased expression of target genes coding for antioxidant enzymes and phase-2-detoxifying enzymes [16, 17, 20]. Antioxidant enzymes include heme oxygenase-1 (HO-1), glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase, whereas phase-2-detoxifying enzymes include NAD (PH): Quinone oxidoreductase-1 (NQO1) and glutathione-S-transferase.

Nrf2 regulates Keap1 levels by controlling its transcription, whereas Keap1 regulates Nrf2 levels by controlling its degradation by proteasome [21]. Immediate early response-3 (IER-3) gene, a multifunctional stress response gene, also regulates Nrf2 activity. Deletion of IER-3 gene increases Nrf2 activity, whereas overexpression of IER-3 decreases it [22].

**EPIGENETICALLY REGULATION OF THE LEVELS OF Nrf2**

The levels of Nrf2 are regulated epigenetically by methylation of CpG (cytosine-phosphate-guanosine) and acetylation of histone3. Hypermethylation of CpG [23] and hyperacetylation of histone3 [24] increase the expression of Nrf2, whereas hypomethylation of CpG and hypoacetylation of histone3 decrease it. Therefore, any agent that can cause hypermethylation of CpG or hyperacetylation of histone3 could be useful in prevention and improved management of PTSD and TBI.

**DIFFERENTIAL RESPONSE OF Nrf2 TO ROS DURING ACUTE AND CHRONIC OXIDATIVE STRESS**

Nrf2 responds to ROS generated during acute and chronic oxidative stress differently. For example, acute oxidative stress during strenuous exercise activates Nrf2 by a ROS-dependent mechanism which translocates from the cytoplasm to the nucleus where it binds with AREs to up-regulate target genes [25]. In other study on rat liver, pretreatment with N-acetylcysteine (NAC) prevents the ROS-induced activation of Nrf2 [26]. Since NAC scavenged all ROS, ROS was not available for activating Nrf2/ARE pathway. This observation should not be interpreted to mean that antioxidant impairs the normal response of activating Nrf2 by ROS. This result suggests that supplementation with a single antioxidant may be sufficient to reduce oxidative damage when consumed immediately after strenuous exercise; however, repeated administration of a single antioxidant during exercise could be detrimental, because the antioxidant would be oxidized under a high oxidative environment and would then act as a pro-oxidant rather than as an antioxidant.

In contrast to acute oxidative stress, Nrf2 becomes unresponsive to ROS during chronic oxidative stress [27-29], suggesting that activation of Nrf2 by a ROS-independent mechanism exists. This is evidenced by the fact that increased chronic oxidative stress occurs in neurodegenerative diseases such as PTSD and TBI. Therefore, identification of agents which can activate Nrf2 by a ROS-independent mechanism would be useful.

**BINDING OF Nrf2 WITH ARE**

An activation of Nrf2 alone is not sufficient to increase the levels of antioxidant enzymes and phase-2-detoxifying enzymes. Activated Nrf2 must bind with ARE in the nucleus for increasing the expression of target genes coding for these cytoprotective enzymes. This binding ability of Nrf2 with ARE was impaired in aged rats and this defect was corrected by supplementation with alpha-lipoic acid [30]. It is unknown whether the binding ability of Nrf2 with ARE is impaired in PTSD and TBI.

**ANTIOXIDANTS REGULATING Nrf2 ACTIVATION AND FREE RADICALS LEVELS**

Antioxidants scavenge free radicals directly at varying levels; however, some can activate Nrf2 by a ROS-independent mechanism as well as scavenge free radicals, while others can activate Nrf2 by a ROS-dependent mechanism. They are described here.

1. **Antioxidants scavenge Free radicals**: All dietary and endogenous antioxidant chemicals reduce varying levels of oxidative stress by directly scavenging free radicals.

2. **Antioxidants which activate Nrf2 by a ROS-independent mechanism and scavenge free radicals**: Some examples are vitamin E and genistein [31], alpha-lipoic acid, [30], curcumin [32], resveratrol [33, 34], omega-3-fatty acids [35, 36], glutathione [37], NAC [38], and coenzyme Q10 [39]. Several plant-
derived phytochemicals, such as epigallocatechin-3-gallate, carestol, kaheol, cinnamonyl-based compounds, zerumbone, lycopene and carnosol [18, 40, 41], genistein [31], allicin, a major organosulfur compound found in garlic[42], sulforaphane, a organosulfur compound, found in cruciferous vegetables,[43], and kavalactones (methylcisin, kavain and yangonin)[44]can activate Nrf2 by a ROS-independent mechanism.

3. Antioxidant which activates Nrf2 by a ROS-dependent mechanism: L-carnitine activates Nrf2 by a ROS-dependent mechanism [45] probably by generating transient ROS.

**REDUCING OXIDATIVE STRESS OPTIMALY**

From the above groups of agents together with other vitamins, a micronutrient cocktail containing dietary antioxidants (vitamins A, C and E, beta-carotene and selenium), polyphenolic compounds (curcumin and resveratrol), endogenous antioxidants (alpha-lipoic acid, L-carnitine, and coenzyme Q10), a synthetic antioxidant N-acetylcysteine (NAC), and omega-3-fatty acids is proposed for optimally reducing oxidative stress by enhancing the levels of antioxidant enzymes and phase-2-detoxifying enzymes through activating the Nrf2/ARE pathway, and multiple dietary and endogenous antioxidant chemicals simultaneously.

**Reducing Chronic Inflammation Optimally**

Activation of Nrf2 also suppresses inflammation [46, 47]. Some individual antioxidant chemicals from the above groups reduce chronic inflammation [2-9]. Therefore, the proposed micronutrient cocktail for reducing oxidative stress may also optimally decrease chronic inflammation.

**REDUCING GLUTAMATE RELEASE OPTIMALLY**

A previous review has discussed in detail involvement of glutamate in PTSD, mild TBI and penetrating TBI [1]. Increased pro-inflammatory stimuli and oxidative stress following TBI cause microglia to release excessive amounts of glutamate, which contributes to loss of neurons [10]. Release of glutamate was blocked by antioxidants, such as vitamin E [10], tempol, a superoxide dismutase mimetic, and edaravone, a synthetic antioxidant [48], quercetin [49], glutathione and vitamin E [50], alpha-lipoic acid [51] and coenzyme Q10 [52]. In addition to antioxidants, Vitamin B-6 [13], vitamin B12 [14] and vitamin B2 (riboflavin) [15]also reduce release of glutamate. Antioxidants such as vitamin E [11] and coenzyme Q10 [12] also protected neurons against glutamate-induced degeneration and death. Therefore, the proposed micronutrient cocktail in combination with B-vitamins may optimally decrease the release of glutamate.

**Nrf2 IN PTSD AND TBI**

Despite potential importance of Nrf2 in reducing the progression and improved management of PTSD and TBI, no studies have been performed on the role of Nrf2 in PTSD, and only a few studies are available in experimental animal models of TBI, using Nrf2 knockout and wild-type mice in which certain antioxidants have been used.

It was demonstrated that activation of NF-kappaB, the levels of pro-inflammatory cytokines, TNF-alpha, IL-1beta and IL-6, and expression of intracellular adhesion molecule 1 (ICAM-1) were higher in the brain of Nrf2 (-/-) knockout mice than in the brain of wild-type Nrf2 (+/+ ) following experimental TBI [53, 54]. These results suggest that the presence of Nrf2 in the neurons is essential for protecting against damage produced by the products of chronic inflammation. The levels of protein carbonyls, 4-hydroxy-2-nonenal (4-HNE) and 8-hydroxy-2-deoxyguanosine (8-OHdG) were higher in the brain of Nrf2 knockout mice (Nrf2-/-) than in the brain of wild-type mice (Nrf2+/+). After brain injury, the levels of antioxidant enzyme heme oxygenase-1 (HO-1) and phase-2-detoxifying enzyme NAD(PH):Quinone oxidoreductase ( NQO1) in wild-type mice increased compared to that in sham-operated one; however, the levels of these enzymes did not significantly change in Nrf2 knockout mice compared to that in sham-operated one [55]. These results suggest that 24 h after brain injury, ROS activated Nrf2 to enhance the transcription of target genes coding for HO-1 and NQO1 in wild-type mice (Nrf2+/+).

Administration of melatonin after experimental TBI reduced oxidative stress, brain edema and cortical neurodegeneration. These protective effects of melatonin were mediated via the Nrf2/ARE pathway, and they were partly abolished in Nrf2 knockout mice [56]. Using subarachnoid hemorrhage (SAH) rat model, it was demonstrated that Nrf2 and its target gene product HO-1 was upregulated in the cortex and peaked at about 24 h after SAH, whereas administration of sulforaphane, which activate Nrf2 [43], 48 hours after SAH reduced early brain edema, blood-brain barrier damage, cortical apoptosis , and motor deficits [57]. Initial activation of Nrf2 after SAH could have been due to the early rise in the levels of ROS. Administration of tert-butylhydroquinone, an activator of Nrf2, after SAH significantly reduced early brain damage (brain edema, blood-brain barrier and cortical apoptosis), behavior and learning deficits [58]. Injection of tert-butylhydroquinone attenuated cerebral...
oxidative stress via the Nrf2/ARE pathway 24 h after brain injury (closed-head brain injury) in mice [59]. Supplementation with sulforaphane, an activator of Nrf2, attenuated brain oxidative stress and neuronal damage after brain injury (controlled cortical impact injury) in rats [60]. Luteolin provided neuroprotection via the Nrf2/ARE pathway in wild type mice (Nrf2+/+), but not in Nrf2 knockout mice (Nrf2-/-) [61].

Hypothermia is used in the management of penetrating TBI including physical injury to the brain. Treatment with mild hypothermia increased the levels of Nrf2mRNA and protein, and translocated Nrf2 from the cytoplasm to the nucleus to upregulate target genes coding for antioxidant enzymes in the cerebral cortex following return of spontaneous circulation in pig model of cardiac arrest [62].

STUDIES ON ANTIOXIDANTS IN PTSD AND TBI

Despite the fact that increased oxidative stress, chronic inflammation and glutamate release play an important role in the initiation and progression of PTSD, mild TBI and penetrating TBI, very little attention has been paid to reducing these biochemical abnormalities. A combination of dietary and endogenous antioxidants and B-vitamins, which may reduce these biochemical defects simultaneously, have not been utilized either in animal or human models of PTSD or TBI. A few studies on the effects of primarily individual antioxidants on PTSD and TBI mostly in animal models are described here.

PTSD

**Individual agents:** The effect of individual agents, such as curcumin [63, 64], flavonoids [65-67] and omega-3-fatty acids [68, 69] were investigated on several criteria in chronically or acutely stressed rats (animal models of PTSD). Different studies measured different criteria. Overall, the results showed that the above treatments somewhat improved cognitive function and neurogenesis in hippocampus, and reduced major depression, bipolar disorder, and impaired feared memory consolidation and reconsolidation processes.

Only one study was available for human PTSD. Daily supplementation with omega-3-fatty acids reduced some of the symptoms of PTSD in critically injured patients during the earthquake in Japan in 2011 [70].

TBI

**Individual Agents:** The effects of individual agents, such as resveratrol, a polyphenolic compound [71-75], alpha-lipoic acid [76, 77], n-acetylcysteine (NAC) [78-81], melatonin [82], vitamin E [83, 84], curcumin [83-86], antioxidant enzymes [87, 88], and omega-3-fatty acids [89-91] have been investigated in animal models of TBI. Different studies measured different criteria, such as markers of oxidative damage and inflammation, edema, blood-brain barrier damage, mitochondrial dysfunction, brain lesion volume, microglia activation, membrane homeostasis impairment, poor motor performance, cognitive dysfunction and glia and neuronal death. Treatment with the above agents individually after TBI reduced somewhat the end-points listed above.

Only two human studies were performed one with individual synthetic antioxidant and one with multiple micronutrients containing dietary and endogenous antioxidants. They are described here.

**Individual antioxidant:** Edaravone, a FDA approved drug, reduced oxidative damage by neutralizing free radicals after TBI in humans [92].

**Multiple micronutrients:** In a pilot study, a commercial formulation of multiple micronutrients was tested in a clinical study in troops returning from Iraq and Afghanistan with mild to moderate TBI. Thirty-four patients with post-traumatic dizziness were admitted to the Naval Medical Center San Diego Clinic over a two-month period of time and agreed to participate in the study under the supervision of Dr. Michael Hoffer and his colleagues [93]. All patients had received their injury 3-20 weeks prior to admission, and they received identical treatment consisting of medical therapy (for any migraines), supportive care, steroids and vestibular rehabilitation therapy. Fifteen of the thirty-four patients also received a dose of an antioxidant and micronutrient formula (two capsules by mouth twice a day). At the onset of therapy all patients were evaluated in outcome measures which included the Sensory Organization Test (SOT) by Computerized Dynamic Posturography (CDP), the Dynamic Gait Index (DGI), the Activities Balance Confidence (ABC) scale, the Dizziness Handicap Index (DHI), the Vestibular Disorders Activities of Daily Living (VADL) score, and the Balance Scoring System (BESS) test. The study was carried out for 12 weeks. The therapist who graded these outcomes and performed the testing was blinded as to whether the patient was receiving antioxidant therapy or not. The pre-trial test scores did not differ significantly between the two groups on any of the tests.

Both groups of patients showed trends toward significant improvement on all tests after the 12 weeks of therapy, but the combination treatment trend was stronger than that of the standard therapy alone group. After only 4 weeks, the SOT score by CDP was 78 for the antioxidant group as compared to 63 for the non-
antioxidant group. This difference was statistically significant at the P < 0.05 level. The improvement noted by the antioxidant group on the other tests was also greater than the non-antioxidant group, although these differences did not reach statistical significance because of the short trial period and small sample size. Nevertheless, these preliminary results suggest that supplementation with a preparation of multiple antioxidants in combination with standard care was more effective than standard care alone in US troops exhibiting the symptoms of mild TBI.

**PROBLEMS ASSOCIATED WITH A SINGLE ANTIOXIDANT IN HUMAN STUDIES**

(1) High risk populations of PTSD, mild TBI have high levels of oxidative stress. Individual antioxidants are easily oxidized; therefore, administered antioxidant would be oxidized under such a high oxidative environment. The oxidized antioxidant acts as a pro-oxidant rather than as an antioxidant; (2) individual antioxidants cannot elevate the levels of antioxidant enzymes and phase-2-detoxifying enzymes by activating Nrf2, and dietary and endogenous antioxidant chemicals simultaneously which is required for optimally reducing oxidative stress, chronic inflammation and glutamate release; (3) distribution of dietary and endogenous antioxidant chemicals differs from one cell to another within the same organ; they also differ from one compartment to another within the same cell. Therefore, supplement with one antioxidant may not reduce oxidative stress throughout the brain; (4) a single antioxidant cannot reduce oxidative stress in both lipid and aqueous environments of the cells; and (5) nicotinamide (B3-vitamins), a competitive inhibitor of histone deacetylase activity, improved mitochondrial function. This function of nicotinamide may not be performed by individual antioxidants.

**Proposed Criteria to be included in the Experimental Designs of Clinical Studies on Antioxidants in Human Studies**

(1) High risk populations, troops in training, ready to be deployed in armed conflicts and athletes of contact sports, such as football, soccer, and ice hockey are suitable populations for prevention study. Troops retuning from armed conflicts and athletes of contact sports who have sustained concussions are suitable for secondary prevention study, whereas veterans of foreign wars, who are on current standard therapy, are suitable for the study on reducing the progression and improved management; (2) experimental designs should be randomized, double-blinded and placebo-controlled; (3) number of participants in the study should be high, generally in hundreds, for a meaningful statistical analysis and conclusions; (4) levels of markers of oxidative stress and chronic inflammation in subset population of smaller number before and after treatments with the proposed mixture of micronutrients should be measured; (5) daily oral supplementation with the proposed mixture of micronutrients that can enhance the levels of antioxidant enzymes and phase-2-detoxifying enzymes through activation of Nrf2, and the levels of dietary and endogenous antioxidant chemicals simultaneously should be included; (6) dose-schedules of twice-a-day should be adopted in order to maintain steady levels of micronutrients; (7) primary end-points, such as development PTSD- or TBI-related symptoms, and dementia should be measured; (8) treatment and observation periods of generally 2-3 years for prevention study, and 3-5 years (for reducing the progression and improving the management studies) are adequate.

Most experimental designs of clinical studies on antioxidants generally included only criteria 1, 2, 3, 7 and 8, but not the criteria 4, 5, and 6.

**PROPOSED PREVENTION STRATEGIES FOR PTSD AND TBI**

**Proposed PTSD Prevention Studies**

**Primary prevention:** The purpose of primary prevention is to protect troops from developing PTSD or TBI. In case of PTSD, it is not feasible to develop primary prevention strategy, because some traumatic events that increase the risk of developing PTSD may occur suddenly. However, in some cases such as troops to be deployed in a military conflict, it is possible to perform primary prevention study. The proposed mixture of micronutrients may be one of the rational choices for primary prevention of PTSD- and TBI-related injuries among troops under training or to be deployed in combat zones. This mixture of micronutrients would enhance the levels of antioxidant enzymes and phase-2-detoxifying enzymes through activating the Nrf2/ARE pathway, and dietary and endogenous antioxidants simultaneously; and thereby, prevent biochemical defects that initiate PTSD- and TBI-related symptoms.

**Secondary prevention:** The purpose of secondary prevention is to stop or slow the progression PTSD or TBI in those individuals who are exposed to blast and other traumatic events, but have not developed clinical signs of PTSD or TBI and are not on specific medications. The proposed mixture of micronutrients suggested for primary prevention can also be used for secondary prevention study. This mixture of micronutrients would enhance the levels of antioxidant enzymes and phase-2-detoxifying enzymes through activating the Nrf2/ARE pathway, and dietary and endogenous antioxidants simultaneously; and thereby,
prevent biochemical defects that contribute to the progression of PTSD- and TBI-related symptoms. Secondary prevention strategy should be implemented at least 24 h after exposure to PTSD- or TBI-related risk factors. This is due to the fact that immediately after exposure to risk factors; both anti- and pro-inflammatory cytokines are released. Anti-inflammatory cytokines help to repair cellular damage. Since antioxidants reduce inflammation, supplementation with antioxidants soon after exposure to risk factors may interfere with the repair processes.

**CURRENT STANDARD THERAPY FOR PTSD**

The purpose of treatment is to slow down the progression of disease and improve the symptoms of the disease. Standard therapy includes drugs and psychological/psychiatric treatment. The drug therapy is primarily based on the symptoms rather than the causes of PTSD. The standard therapy has produced very limited success in the treatment of PTSD. None of the drugs used in the treatment of PTSD affect the levels of increased oxidative stress, chronic inflammation and glutamate release that play an important role in the progression of PTSD. Therefore, additional approaches that could improve the management of PTSD and reduce the progression of the disease should be developed. Some examples of commonly used medications to improve the symptoms of PTSD are described below. The detail information can be found in the website of United states Department of Veterans Affairs (National Center for PTSD, 2013). The groups of drugs include Selective serotonin reuptake inhibitors (SSRIs), such as Zoloft, Paxil, and Prozac, antidepressants, such as Remeron, Effexor, and Serzone, mood stabilizer drugs, such as Tegretol, Depakote, Lamictal, Topimax, Minipress, tricyclic antidepressants imipramine and monoamine oxidase inhibitors (Phenelzine), benzodiazepines, such as Ativan, klonopin and Xanax, d-serine, cycloserine and cortisol.

These medications did not reduce oxidative stress, chronic inflammation and glutamate release that contribute to the initiation and progression of PTSD. Consequently, neurons continue to die despite drug treatment. Therefore, these biochemical defects must be simultaneously attenuated while undergoing standard therapy. This is essential in order to reduce the progression and improve the current management of PTSD.

**CURRENT THERAPY FOR TBI**

Individuals who sustain penetrating TBI provide an excellent opportunity to study the efficacy of a mixture of multiple micronutrients, in combination with standard therapy, in reducing the secondary damage during the acute and chronic phases of injury. These patients require immediate emergency care in the hospital to receive standard therapy in order to stabilize their conditions.

The penetrating TBI is extremely difficult to treat, because of the inherent complexity of the brain structures and functions as well as extreme variations in the pattern of injury. Approximately half of severely brain-injured patients require surgery to remove or repair hematomas (rupture of blood vessels) or contusions (bruised brain tissue) (National Institute of Neurological Disorders and Stroke, 2009). Initial treatment focuses on preventing secondary injury following TBI. This includes proper oxygen supply to the brain and the rest of the body, maintaining adequate blood flow, and controlling blood pressure.

Hypothermia (32-33°C) has been used in the management of TBI. In a clinical study, it was demonstrated that hypothermia attenuated the levels of markers of oxidative stress in infants and children after penetrating TBI [94]. The effect of hypothermia was analyzed in 12 studies with 1,327 patients in whom 8 studies implemented hypothermia according to a long-term or goal-directed strategy, and 4 studies implemented hypothermia according to a short-term strategy. The results revealed that when only a short-term strategy of hypothermia was performed, neither mortality nor neurological outcomes were improved; however, when a long-term hypothermia strategy was used, mortality was reduced and neurological outcomes were improved [95]. The optimal results were obtained when cooling was continued for at least 72 hours and/or until intracranial pressure was normalized for at least 24 hours. In children with penetrating TBI, the cerebral spinal fluid (CSF) levels of alpha-synuclein were elevated in patient with TBI than in control subjects; however, the CSF levels of alpha-synuclein decreased after hypothermia treatment [96].

Medications to reduce secondary damage to the brain immediately after injury may include diuretics in order to reduce pressure from the brain by eliminating fluid through urine, anti-seizure drugs during the first week to avoid additional damage to the brain that might be caused by seizures, and coma-inducing drugs. In addition, emergency surgery may be needed to remove blood clot, repair skull fractures, and open a window in the skull to relieve pressure inside the brain by draining accumulated fluid. Patients with penetrating TBI receive rehabilitation therapy that includes individually tailored treatment program in the area of physical therapy, occupational therapy, speech/language therapy, medications, psychology/psychiatry therapy, and social support. The standard therapy has markedly
improved the management of penetrating TBI and has increased the survival rate.

The current standard therapy does not decrease oxidative stress, inflammation and glutamate release that contribute to the development of secondary injury to the brain during acute phase, and to the development of late adverse effects during chronic phase of injury. Consequently, neurons continue to die despite standard therapy. These biochemical defects must be simultaneously attenuated while undergoing current therapies. This is essential in order to reduce the progression and improve the current management of penetrating TBI.

Proposed micronutrient cocktail in combination with drug therapy: The micronutrient cocktail recommended for primary prevention can also be used in combination with standard drug therapy for both PTSD and TBI. This micronutrient cocktail would enhance the levels of antioxidant enzymes and phase-2-detoxifying enzymes through activating the Nrf2/ARE pathway, and dietary and endogenous antioxidants simultaneously; and thereby, reduce the biochemical defects that contribute to the progression of PTSD- and TBI-related symptoms. This strategy may reduce the progression and prolong the beneficial effects of current drugs in patients with PTSD and TBI by protecting neurons from damage produced by increased oxidative stress, chronic inflammation and glutamate release.

SAFETY OF INGREDIENTS IN THE PROPOSED MIXTURE

All ingredients in the proposed mixture of micronutrients are considered safe. They come under category of “Food Supplement”, and therefore, do not require FDA approval for their use. A few of them could produce harmful effects at high doses in some individuals when consumed daily over an extended period. Vitamin A at doses of 10,000 IU or more per day can cause birth defects in pregnant women, and beta-carotene at doses 50 mg or more can produce bronzing of the skin that is reversible on discontinuation. Vitamin C as ascorbic acid at high doses (10 grams or more per day) can cause diarrhea in some individuals. Vitamin E at high doses (2,000 IU or more per day) can induce blood clotting defects after long-term consumption. Vitamin B6 at high doses (50 mg or more per day) may produce peripheral neuropathy, and selenium at doses 400 mcg or more per day can cause skin and liver toxicity after long-term consumption. Coenzyme Q10 at daily doses of 30-400 mg has no known toxicity. N-acetylcysteine doses of 250-1500 mg and alpha-lipoic acid doses of 600 mg are used in humans without reported toxicity.

In conclusion, increased oxidative stress, chronic inflammation and glutamate release play a central role in the initiation and progression of PTSD and TBI in humans. The proposed micronutrient cocktail containing dietary and endogenous antioxidant chemicals, curcumin, resveratrol, B-vitamins, vitamin D, and omega-3-fatty acids may increase the levels of antioxidant enzymes and phase-2-detoxifying enzymes by activating a nuclear transcriptional factor Nrf2 and dietary and endogenous antioxidants simultaneously. This micronutrient cocktail may reduce the incidence and progression of PTSD and TBI. The same micronutrient cocktail, in combination with the standard therapy, may improve the current management of these neurological disorders.

Conflicts: The author owns stocks in Premier Micronutrient Corporation.

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