Short Communication

An H_2-infused, nitric oxide-producing functional beverage as a neuroprotective agent for TBIs and concussions

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Traumatic brain injuries (TBIs) are a leading cause of death and disability. Sports-related TBIs are estimated to be more than several million per year. The pathophysiology of TBIs involves high levels of inflammation, oxidative stress, dysregulation of ion homeostasis, mitochondrial dysfunction, and apoptosis. There is also a reduction in cerebral blood flow, leading to hypoxia and reduced removal of metabolic waste, which further exacerbates the injury. There is currently no recognized effective medical treatment or intervention for TBIs, which may in part be due to the difficulty of drug delivery through the blood-brain barrier. Molecular hydrogen has recently emerged as a neuroprotective medical gas against cerebral infarction and neurodegenerative diseases including TBIs. Its small molecular size and nonpolar nature allow it to easily diffuse through the blood-brain barrier, cell membranes and subcellular compartments. Hydrogen has been shown to exert selective anti-inflammatory, antioxidant, and anti-apoptotic effects by regulating various transcription factors and protein phosphorylation cascades. Nitric oxide is another well-recognized medical gas that plays divergent roles in protecting from and in the recovery of TBIs, as well as in contributing to their pathophysiology and injury. Excessive activation of inducible nitric oxide synthase leads to excess inflammation and oxidative/nitrosative damage as well as a paradoxical nitric oxide depletion in the locations it is needed. Hydrogen regulates nitric oxide production and metabolism, which enhances its benefits while reducing its harms. A novel H_2-infused, nitric oxide producing beverage, Hydro Shot, may have important neuroprotective benefits for TBIs. We report preliminary indications that Hydro Shot may be a meaningful adjuvant treatment for TBIs.

Keywords

Traumatic brain injury; Molecular hydrogen; Nitric oxide; Inflammation; Reactive oxygen specie

1. Introduction

Traumatic brain injuries represent a serious health concern for millions of people and are a contributing risk factor in the development of other neurodegenerative diseases. However, there are many obstacles that need to be overcome in order to address these brain injuries including diagnosis, treatment, and monitoring. This article briefly summarizes the current status and problems with diagnosing, monitoring and treating traumatic brain injuries as well as its molecular pathophysiology. We introduce a novel approach for the treatment of traumatic brain injuries using medical gases, nitric oxide and molecular hydrogen. The scientific literature of both of these gases is briefly reviewed followed by some preliminary data in which administration of both gaseous molecules through a novel functional beverage improves cognitive function.

2. TBIs, concussions, and chronic traumatic encephalopathy (CTE)

A traumatic brain injury (TBI) occurs when the brain is injured by an external source from a significant acceleration or deacceleration, impact, or blast wave. TBIs are a leading cause of death and disability worldwide [1]. Sports-related TBIs make up to one-third of all causes of TBIs [2]. Mild TBIs are called concussions and are the most common types of TBIs. The Center of Disease Control estimates that there are around 300,000 sports-related concussions per year in the USA [3]. However, this number only includes athletes who have lost consciousness, which occurs in only about 10% of concussions [4]. The real number may be more than several million per year [5]. The vast majority of people who suffer a TBI do not immediately die from the primary injury, i.e., the damage directly to the brain tissue and blood vessels. Instead, most consequences occur from the secondary injury largely due to excessive inflammation and production of reactive oxygen species.

The secondary injuries may result in post-concussion syndrome in which the symptoms continue to last for months to

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more than a year after a TBI. Frequent TBIs increase the risk for developing the neurogenerative disease, chronic traumatic encephalopathy [6]. It normally takes eight to ten years after repetitive concussions for the stages of CTE to appear. The symptoms of these stages include confusion, headaches, memory loss, impulsive behavior, depression, suicidality, etc. [6].

3. Diagnosis and testing for TBIs

Diagnosis of TBIs may include neuroimaging such as a computed tomography (CT) scan, functional magnetic resonance imaging (fMRI), single photon emission computed tomography (SPECT), and positron emission tomography (PET) scans. However, most concussions are not usually associated with visible lesions that can be detected by current imaging techniques [7]. Unfortunately, there is no urine, saliva, or blood test that can confirm either the existence of a TBI or recovery from a TBI [8]. Accordingly, cognitive functional assessments are imperative to understanding the prognosis and recovery of TBIs [7]. The most widely used psychometric test is the "Immediate post-concussion assessment and cognitive testing" abbreviated as "ImPACT" [9]. Another method that can be useful is a neuro-diagnostic called Brain Gauge by Cortical Metrics. This is also an FDA-approved device that has been demonstrated to be an effective tool for monitoring TBIs. It measures eight essential components of brain health including, speed, focus, fatigue, accuracy, sequencing, timing, perception, plasticity, and connectivity [10].

In one study, over 200 student athletes were tracked post-concussion with the Brain Gauge and the results were compared to other methods including ImPACT and balance testing, and it was found that the Brain Gauge was highly sensitive and accurate in the measurements. This is largely because of the high precision and accuracy of measuring reaction time, which is an objective indicator of concussions and recovery [11]. The precision (0.33 milliseconds) is approximately 1000× more precise than visual reaction time methods. The Brain Gauge has been validated by over 50 years of clinical studies and is currently used by more than 50 universities around the world. It is also used by the Office of Naval Research and Applied Research Associates to help improve diagnosis and treatment of military personnel exposed to blasts. Accordingly, in addition to conventional ImPACT testing fMRI, CT and PET scans, the Brain Gauge may represent an important tool for concussion diagnostics and to monitor improvements in cognitive function over time.

4. Molecular pathophysiology of TBIs

Despite having a mass of only 2% of the body, the brain constitutes about 20% of the normal resting metabolic rate. In other words, the brain consumes about 20% of the available oxygen in order to maintain normal cognitive function [12]. In order to receive this much oxygen, the brain receives a high proportion (≈15%–20%) of the blood from cardiac output [13]. However, after adolescence, cerebral blood flow declines with normal aging at around 0.5% a year [14]. As a logical consequence of the decreased blood flow are the impairments to cognitive function and increased dementia that similarly increase with aging [15]. Additionally, middle-aged subjects with metabolic syndrome have decreased cognitive function and 15% lower blood flow compared to their healthy age-matched counterparts [16].

As expected, traumatic brain injuries (TBIs) also dysregulate and impair cerebral blood flow [17]. Normally cerebral autoregulation maintains adequate brain perfusion during systemic changes in blood pressure. However, TBIs impair the brain's autoregulation of blood flow, which is correlated with poor cognitive function [17]. The decreased cerebral blood flow predicts the severity, cognitive dysfunction, and recovery from a TBI [18]. The reduced brain blood flow means a reduction in oxygen and glucose, which leads to a pathological energy crisis [19]. Reduced oxygen impairs mitochondrial activity, thus decreasing adenosine triphosphate (ATP) from oxidative phosphorylation. Under low oxygen conditions, anaerobic glycolysis would normally increase to ensure sufficient ATP; however, although glycolysis increases, the lower blood supply limits glucose availability resulting in further reductions in ATP and subsequent neuronal dysfunction [20].

The decrease in ATP production decreases Na^+/K^+ ATPase pump activity resulting in membrane depolarization and dysregulation of potassium, calcium, and sodium ions. As a consequence, high levels of the excitatory neurotransmitter glutamate are released resulting in excessive neuronal stimulation leading to more ion imbalances. This is called glutamate excitotoxicity since the process is toxic to the cells and leads to cellular death [21]. The increased calcium levels directly stimulate apoptotic pathways resulting in more cell death. Calcium also activates various inflammatory transcription factors such as the proto-oncogene c-FOS, (nuclear factor of activated T-cells) NFAT, (nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), toll like receptor 4 (TLR4), high-mobility group box 1 (HMGB1), protein kinase C (PKC), and inducible nitric oxide synthase (iNOS). The increased inflammation further triggers neuronal apoptosis. Additionally, the ion imbalances, high inflammation, and activation of protein kinase C and iNOS result in over production of reactive oxygen species leading to direct damage to deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, and cell membranes, which further increases inflammation, mitochondrial dysfunction, and cell death. The pathological situation worsens as the blood flow decreases further. This not only impairs oxygen and substrate availability, but also the ability to remove cellular metabolic waste including excess neurotransmitters and inflammatory mediators. Fig. 1 provides a recapitulation of primary and secondary TBI pathology and related sequala along with various proposed interventions.

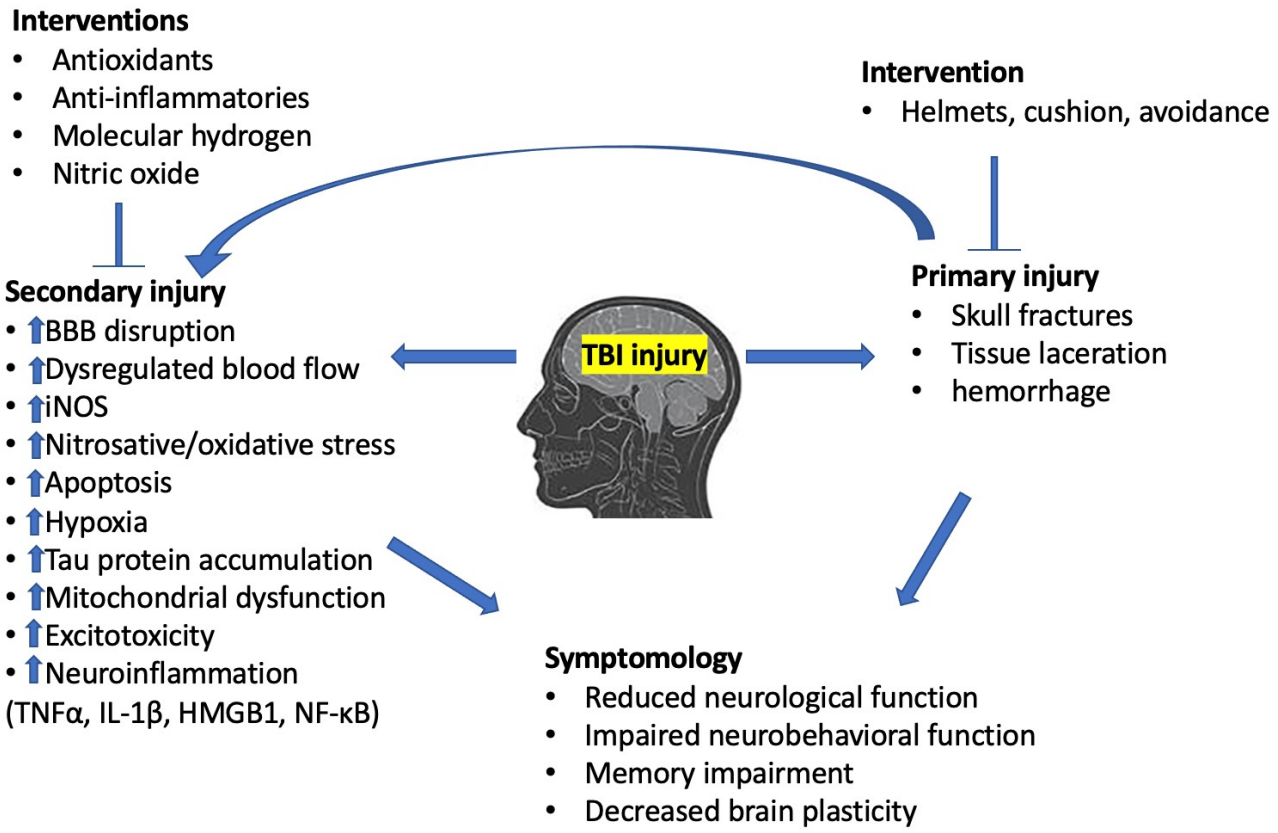

Fig. 1. Pathophysiology of TBI injury and related consequences and interventions.

5. Treatment for TBIs

Immediate treatment of a TBI is imperative to help reduce the secondary injuries previously discussed. However, there is no known effective treatment or medications for TBI. Most recommendations simply include physical rest while slowly transitioning from light work back to normal activity once it appears that cognitive function has been restored [8]. However, this does not address the pathological cascades resulting in secondary brain injury such as oxidative stress, inflammation, reduced blood flow, metabolic dysregulation, glutamate excitotoxicity, and neuronal apoptosis. Conventional antioxidants and anti-inflammatories are often ineffective at preventing or treating human diseases [22], but still may have some benefit for TBI [23].

There have been many preclinical studies in various animal models using different bioactive molecules. For example, in a mouse model of TBI-induced by controlled cortical impact, treatment with *Coriolus versicolor* and *Hericium erinaceus* from mushrooms restored behavioral alterations and decreased the neuroinflammatory and oxidative stress [24]. In a similar TBI-mouse model, administration of artesunate from the Chinese plant *Artemisia annua* was able to favorably modulate neurotropic factors and suppress NF-κB and the nucleotide-binding oligomerization domain, leucine-rich-containing family, pyrin domain-containing 3 (NLRP3) inflammasome complex [25]. However, despite a variety of animal models of TBIs [26], there is a fundamental disconnect between these studies and successful translation to treating people for TBIs [27]. One of the challenges with treating TBIs with pharmaceuticals/nutraceuticals is that they do not easily penetrate the blood-brain barrier because of their size, charge, and polarity [28].

6. Molecular hydrogen

Molecular hydrogen (H_2 gas) has recently emerged as an ergogenic and medicinal therapeutic for exercise performance [29] and clinical medicine [30], respectively. Hydrogen is the smallest molecule in the universe with no charge or polarity, which gives it the fastest rate of diffusion and easy penetration through cell membranes, including the intracellular compartments such as the mitochondria and nucleus, and easily diffuses through the blood-brain barrier [30]. Hydrogen was first shown to be neuroprotective in 2007, when an article published in *Nature Medicine* demonstrated that H_2 could markedly suppress brain damage in a rat model of cerebral infarction induced by a middle cerebral artery occlusion [31]. The article further demonstrated that H_2 acts as a selective antioxidant in that it only reduces the highly toxic oxidants and does not neutralize the beneficial signaling molecules [31]. Since then, around 2000 publications and over 100 human clinical studies have added credence to the therapeutic effects of H_2. However, the exact primary targets

remain elusive, and the main mechanisms have largely been attributed to hydrogen's unique selective antioxidant, anti-inflammatory, and cell-signal modulating activities.

Hydrogen administration for TBIs and concussions

The chemical properties and biological effects of hydrogen make it an attractive molecule for the treatment of TBIs. There are many therapeutic effects of H_2 that could feasibly be of benefit in protecting against TBIs, including protection against glutamate-induced excitotoxicity [32], regulation of aberrant calcium signaling [33], inhibition of mitochondrial permeability transition pores [34], suppression of proinflammatory cytokines and proteins that are often expressed in TBIs (e.g., cFOS, NFAT, NF-κB, TLR4, HBMG1, NLRP3, protein kinase C, etc.) [30], and other conceivable benefits as has been recently reviewed [35]. A number of studies have specifically investigated the effects of H_2 administration on TBIs and reported favorable results. For example, a study from University of Washington found that drinking hydrogen water was protective against the neurodegenerative changes induced by a TBI [36]. They used an experimental model of controlled cortical impact, which induced significant neuropathological aberrations. However, ingestion of hydrogen-rich water reversed TBI-induced edema by $\approx$ 50%, completely blocked pathological tau expression, attenuated inflammatory cytokines, reversed the pathological changes in aquaporin-4, HIF-1, MMP-2, and MMP-9. Moreover, it was found that hydrogen treatment either increased or preserved the mitochondrial ATP levels independent of the electron transport chain [36]. Similarly, another group using a rat model (n = 50) of free-fall impact found that H_2 significantly improved the neurological severity score compared to the TBI group without hydrogen. Mechanistically it was found that expression of inflammatory markers tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), reactive oxygen species (ROS) levels, and markers of apoptosis all significantly decreased [37]. Correspondingly, another report found that H_2 significantly increased the 7-day survival time and reduced neurological deficits [38]. This was mediated by H_2-enhanced translocation of cytoprotective Nrf2 protein into the nucleus resulting in decreased oxidative stress [38]. Table 1 (Ref. [36, 38–50]) provides a summary of the symptomatic, cellular, and molecular benefits of H_2 on TBIs and concussions.

In addition to the studies directly using H_2 to treat TBIs, there are also supportive clinical studies on the use of H_2 for other brain injuries that share common pathologies. For example, human clinical studies have demonstrated that hydrogen improves mild cognitive impairments [51], cerebral infarction [52], and improves mood and anxiety in healthy subjects [53]. As mentioned earlier, subjects with metabolic syndrome tend to have lower cerebral blood flow [16], and several clinical studies have demonstrated that drinking hydrogen water can improve this condition [54–56]. Another human study in newborn infants (n = 40) that suffered from reduced cerebral blood flow were administered hydrogen water two days after birth daily for ten days [57]. Hydrogen water was shown to be safe and lowered the inflammatory markers TNFα and IL-6 and significantly decreased neuron–specific enolase, a sensitive marker for nerve cell damage [57].

7. Role of nitric oxide in TBIs and concussions

Another medical gas that has shown therapeutic effects for TBIs is nitric oxide ($NO^{\bullet}$) [58]. Nitric oxide is primarily produced via nitric oxide synthase (NOS) enzymes, of which there are three isoforms, namely, inducible NOS (iNOS), neuronal NOS (nNOS), and endothelial NOS (eNOS) [59]. Nitric oxide levels gradually decrease with age; a 70-year-old may have a 75% reduction in nitric oxide levels compared to a healthy 20-year-old [60]. This may help explain why cerebral blood flow also decreases with age [14, 15]. One principal reason why nitric oxide can improve TBI is because it is able to increase cerebral blood flow, which is significantly reduced following a TBI [17]. Accordingly, nitric oxide also has many beneficial effects if its production and metabolism are regulated and in the desired locations where it can increase the needed blood flow [58]. One study found that when nitric oxide was administered post-TBI via inhalation in mice, there was a significantly reduced lesion volume and brain edema with less disruption to the blood-brain barrier and improved neurological function [61]. Similarly, another group reported that nitric oxide administration significantly protected against TBI-induced neuronal cell necrosis and maintained cerebral autoregulation of blood flow in juvenile pigs [62].

Pathological effects of nitric oxide

However, nitric oxide also has a dark side as its dysregulation mediates many of the pathological consequences of TBIs. A decreased eNOS activity results in decreased blood flow and subsequent reductions in oxygen and glucose availability, and decreased removal of metabolic waste and cellular debris. The increased inflammation leads to an increased iNOS activation, which is expressed in macrophages and glial cells [63]. Unlike eNOS, increased iNOS expression is often harmful and contributes to the pathophysiology of secondary TBI injuries [63]. The high levels of $NO^{\bullet}$ increase the reactions with superoxide, which forms peroxynitrite ($ONOO^{-}$). This highly oxidative molecule damages mitochondria, the vascular endothelium, and smooth muscle cells [64]. Additionally, the high iNOS activity results in local substrate depletion for eNOS activity, paradoxically resulting in $NO^{\bullet}$ depletion [58].

In humans suffering severe TBI, the levels of arginine and citrulline were significantly reduced compared to the control group [65]. Accordingly, increasing or preserving arginine availability is paramount in improving the prognosis of TBIs. This can be attenuated by increasing arginine availability via supplementation [66]. Supplementation with cit-

Table 1. Benefits of H_2 for TBIs and concussions.

Phenotypic, cellular and mechanistic effects of H_2 on TBI/concussions	Reference
Symptomatic/phenotypic benefits	
• Improved neurofunctional outcome	[39]
• Improved neurological severity score	[38]
• Improve neurological function	[40, 41]
• Improved neurobehavioral function	[41]
• Prevention of neurological dysfunction	[42]
• Reduced neurological deficits	[38, 43]
• Improved cognitive performance and brain plasticity	[44]
• Increased 7-day survival rates	[38]
Cellular/tissue benefits	
• Reduced brain edema	[41–46]
• Reduced lesion volume	[40]
• Reduced tissue damage/cell death	[39]
• Reduced blood-brain barrier permeability/disruption	[40–42]
• Increased angiogenesis/capillarization	[46]
Molecular benefits	
• Reduced mast cell activation	[41]
• Reduced microglia activation and neuroinflammation	[47]
• Reduced apoptosis (increased Bcl-2/Bax ratio, and decreased caspase-3)	[39, 43, 48]
• Reduced aquaporin expression	[36, 45]
• Reduced pathological tau expression	[36]
• Reduced silent information regulator 2 (sir2)	[44]
• Reduced inflammation (e.g., TNFα, IL-1β, HMGB1)	[36, 42, 48]
• Reduced reactive oxygen species	[40, 43, 44, 48]
• Increased antioxidant status	[40, 41, 48]
• Increased brain-derived neurotropic factor	[44]
• Increased ATP levels	[36]
• Regulation of autophagy activation	[49, 50]

Bcl-2, B-cell lymphoma protein 2; Bax, Bcl2 associated X-protein.

rulline is more effective at increasing plasma arginine and $NO^{\bullet}$ levels compared to supplementing with arginine [67]. Low levels of citrulline may also lead to NOS uncoupling, which induces further oxidative and cellular damage [68].

8. Molecular hydrogen and nitric oxide combination

The novel combination of these two medical gases has important clinical implications [69]. For example, molecular hydrogen is able to attenuate the aforementioned dark side of nitric oxide and regulate its production and metabolism. This includes (i) enhanced expression of protective eNOS, (ii) decreased inflammatory mediators that impair $NO^{\bullet}$ signaling, (iii) decreased pathological iNOS activity, (iv) reductions in $ONOO^{-}$ formation, and (v) protection against $ONOO^{-}$ as evidenced by the elimination of nitrotyrosine levels when the two gases were administered simultaneously [70]. Additionally, H_2 can enhance the benefits of $NO^{\bullet}$ by regulating its production and increasing its circulating half-life while also enhancing its therapeutic effects [71].

9. Methods and results for a novel $H_2/NO^{\bullet}$ functional beverage

In order to evaluate the potential of an $H_2/NO^{\bullet}$ combination on cognitive function using neurocognitive assessments, we used a beverage called Hydro Shot. This is a zero-calorie, functional beverage containing nitric-oxide-stimulating citrulline that has been infused with molecular hydrogen. The concentration of H_2 is above 2 mg/L as determined via gas chromatography (H_2 Analytics, Las Vegas, USA; SRI 8610C; California, USA). All ingredients have a high safety profile and are designated as GRAS (Generally Recognized as Safe) by the USFDA. It was previously reported [71] that this novel H_2-infused, nitric oxide-producing functional beverage significantly increased nitric oxide production and blood flow [72] with a corresponding increase in exercise performance and cognitive function [71]. Here, we briefly report the effects of Hydro Shot on various cognitive indices using different neurocognitive testing methods as explained below that are used to indicate the presence of TBIs and concussions. The cognitive function of six male subjects (>65 years) was tested. All tests were carried out in an open label, non-blinded fashion Since results were similar, we presented the computer-generated graphics for a 74-year-old male. Due to

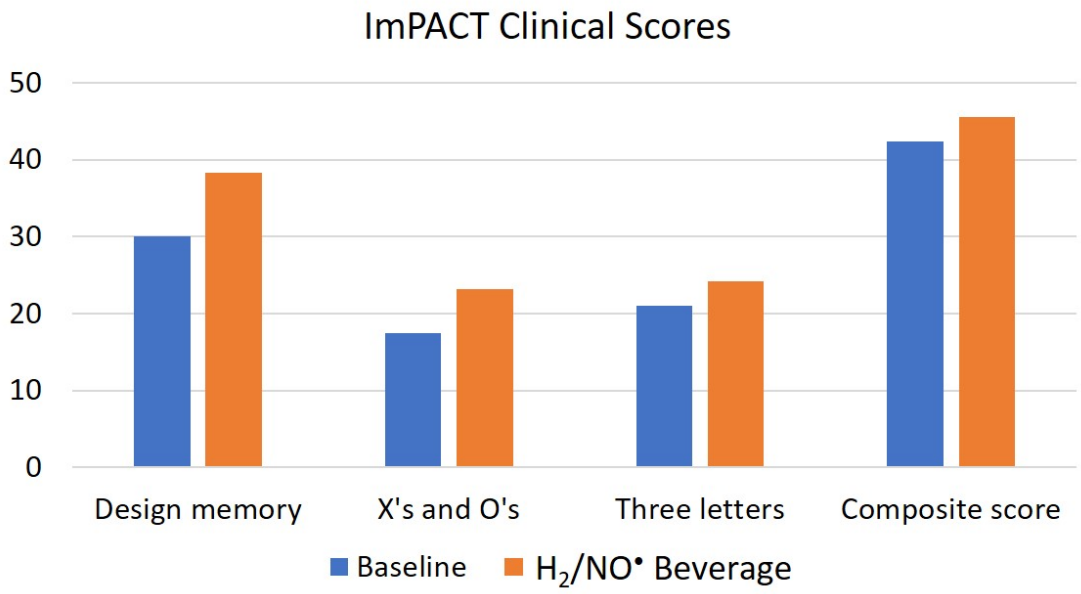

Fig. 2. Effects of functional beverage on clinical ImPACT scores. Ingestion of beverage improved ImPACT scores compared to baseline.

the sample size and methodology of this study, caution should be employed when interpreting these preliminary results.

9.1 H_2/NO• beverage improves clinical ImPACT scores

In order to assess the effects of the functional beverage on cognitive function related to TBIs, the FDA-approved medical device, ImPACT was used (ImPACT Applications, Inc., Coralville, IA, USA) As mentioned, this widely used computer software gives a battery of neurocognitive tests for concussion care. A clinical ImPACT report was generated, and the average value of each category was determined. The composite score consisted of five parts (memory verbal, memory visual, visual-motor speed, reaction time, and impulse control). These values were totaled to find the average so that each category as a whole can be compared directly to each other. Fig. 2 shows the results of completing the assessment with and without Hydro Shot. As noted in the figure, this novel functional beverage improved the ImPACT clinical scores indicating its ability to improve functional cognition.

9.2 Hydro shot improves cognitive scores using brain gauge

It was previously reported that Hydro Shot significantly improved cognitive function as assessed by Brain Gauge (Cortical Metrics, Chapel Hill, NC, USA) [71]. As discussed earlier, the Brain Gauge cognitive assessment tool has been validated by many studies and serves as an important indicator of TBIs. Fig. 3 illustrates the effects of the novel functional beverage on cognitive function using a radar chart.

The H_2/NO• beverage induced significant improvements in the multivariant cognitive indices as illustrated in the radar chart. The scores were higher and also more symmetrical and balanced. These results indicate that this novel beverage can improve cognitive function as assessed by neuropscyometric testing.

10. Discussion

TBIs are a leading cause of death and disability worldwide [1]. Various diagnostic and assessment tools are available including neuroimaging techniques (e.g., fMRI, CT and PET scans, etc.), and computer software neuropsychometric testing. However, neuroimaging methods are expensive, time consuming, and do not always detect the presence of existing TBIs. The neuropsychometric assessments, such as ImPACT and Brain Gauge, appear to be more accurate in diagnosing and monitoring TBIs. Brain Gauge has a high level of sensitivity, accuracy, and precision for assessing cognitive function. We report that Hydro Shot significantly improved the clinical scores using ImPACT testing as well as the Brain Gauge. The significant improvements from Hydro Shot may be due to its ability to increase nitric oxide production and subsequent cerebral blood flow as well as the neuroprotective effects of molecular hydrogen.

The level of cerebral blood flow may predict the recovery, injury severity and cognitive function from TBIs. The reduction in cerebral blood flow is largely due to reductions in nitric oxide. Nitric oxide plays a key role in the prognosis, recovery, and pathogenesis of TBIs. Molecular hydrogen also has important benefits for TBIs and concussions at improving neurological outcomes and reduced brain damage. These benefits are primarily due to the unique properties of hydrogen including (i) rapid diffusion and penetration through all cell membranes and subcellular compartments (e.g., nucleus, mitochondria, and blood-brain barrier), (ii) selective antioxidant activity, (iii) selective anti-inflammatory activity, (iv) prevention of pathological apoptosis, (v) favorable effects on microRNA and gene expression, (vi) improved mitochondrial function, (vii) regulation of nitric oxide production and metabolism, (viii) enhancement of nitric oxide's therapeutic

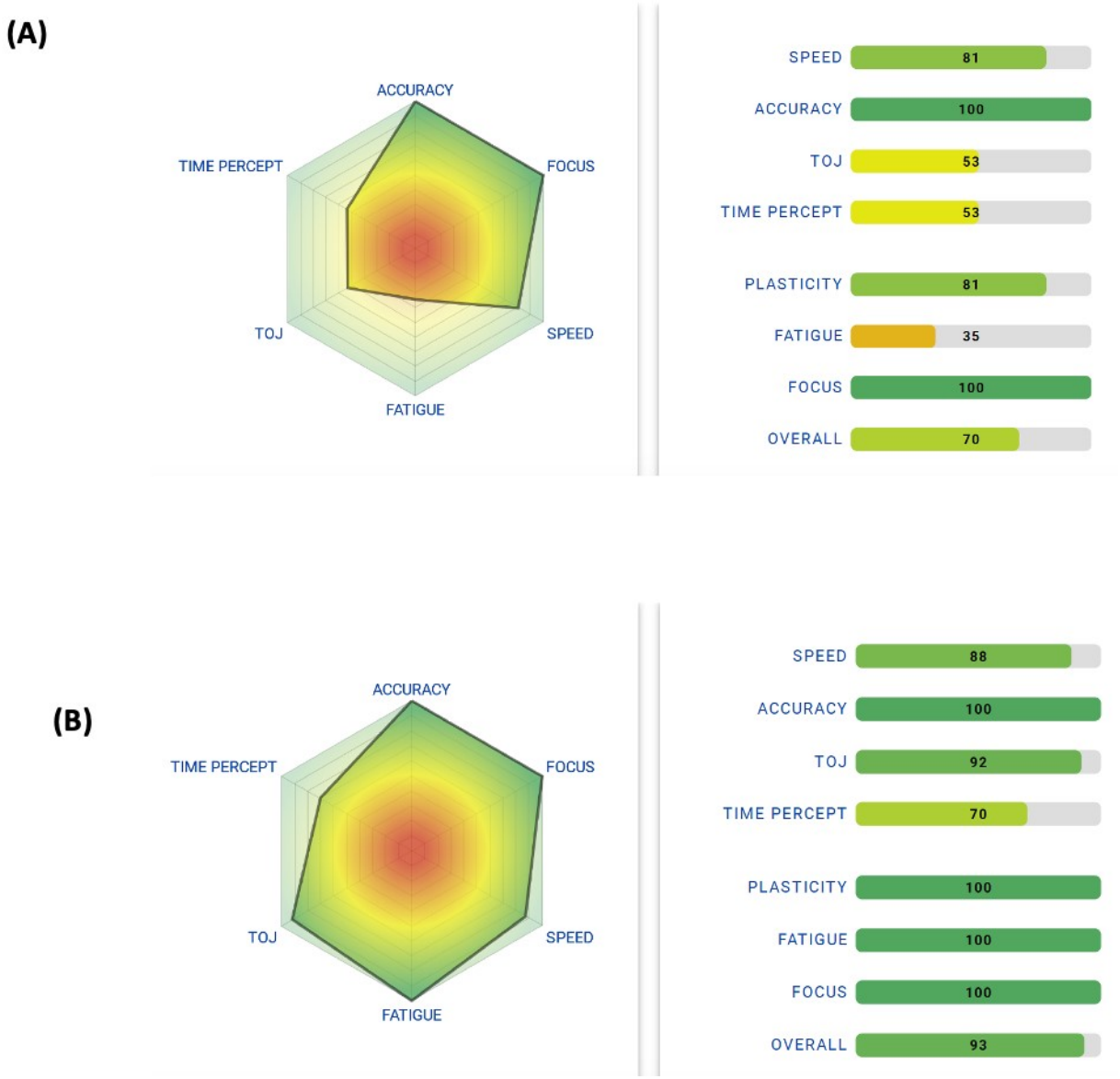

Fig. 3. Radar charts of cognitive function. (A) Without beverage. (B) With beverage. Cognitive scores were significantly improved above baseline following ingestion of the novel beverage.

effects, (ix) suppression of excess iNOS activity, and (x) protection against nitrosative stress induced by $ONOO^-$.

The cellular damage induced by TBIs is due to both primary and secondary injuries to the brain. Prevention is the best strategy for primary injuries, but once it occurs, then treatment of secondary injuries is paramount. However, no approved or effective medical treatments for TBIs currently exist. The secondary injuries occur due to increased oxidative stress, inflammation, and impaired metabolism largely attributed to reductions in cerebral blood flow, which decreases glucose and oxygen availability. Cerebral blood flow also declines with age in accordance with the decline in nitric oxide levels. Physical exercise and healthy dietary choices increase global and regional cerebral blood flow and improve cognitive functioning [13]. The studies on the effects of molecular hydrogen and nitric oxide make it an attractive combination for TBIs and concussions.

11. Conclusions

Molecular hydrogen has emerged as an important medical gas with favorable physicochemical properties that make it ideal for the treatment of secondary TBIs. This is supported by pre-clinical studies and relevant human clinical studies. Similarly, nitric oxide has essential biological effects to treat TBIs but not without undesirable side effects. The novel approach of combining molecular hydrogen with nitric oxide may significantly improve the prognosis of TBIs by exerting individual and potential synergistic therapeutic effects with hydrogen mitigating nitric oxide toxicity.

The preliminary results obtained with the novel $H_2/NO^{\bullet}$

functional beverage, Hydro Shot, indicate that such an intervention may offer improvements in not only physical exercise, but also exert neuroprotection against a variety of traumatic brain injuries. However, well-designed, placebo-controlled clinical studies are strongly warranted to further investigate the potential use of the novel and simple approach of using these two medical gases (H_2 and $NO^{\bullet}$) administered via this unique beverage formulation. It is also recommended that neuroimaging technology be employed to determine the effects of this $H_2/NO^{\bullet}$ combination on cerebral blood flow, oxygen concentration, and cerebral activity. Additionally, *in vitro* studies are encouraged to elucidate the molecular mechanisms responsible for the observed therapeutic effects of this novel approach.

Abbreviations

CT, computed tomography; CTE, chronic traumatic encephalopathy; eNOS, endothelial nitric oxide synthase; HIF-1, hypoxia inducible factor; IL-1β, interleukin-1 beta; IL-6, interleukin 6; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NFAT, nuclear factor of activated T cells; nNOS, neuronal nitric oxide synthase; Nrf2, nuclear factor erythroid 2-related factor 2; PKC, protein kinase C; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen species; TBI, traumatic brain injury; TLR4, Toll-like receptor 4; HMBG1, high mobility group box 1 protein; TNFα, tumor necrosis factor alpha.

Author contributions

TWL, JK, MLM conceived and designed the experiments; JK and MLM performed the experiments; TWL and JK analyzed the data; TWL and JK wrote the manuscript. All authors approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

MLM is a scientific advisor to the company. TWL reports personal fees from medical/academic conferences including travel reimbursement, honoraria, and speaking and consultancy fees from various academic and commercial entities regarding molecular hydrogen. All other authors report no conflict of interest.

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