A Theory on The Mechanisms of Traumatic Brain Injury and a Potential Treatment Protocol

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Abstract

Traumatic brain injury (TBI) is a leading cause of death and disability in the United States[8]. TBI's mechanisms of action include both the initial sheering and stressing of axons, caused by various physical insults to the brain, as well as a cascade of secondary bio-chemical injuries. The persistent secondary chemical injuries present in TBI continue a cycle of cellular damage which leads to eventual cellular apoptosis[5,18]. This cycle of cellular damage is created and sustained by the brain's microglial cells. We theorize that the chronic activation of microglial cells, microglial priming, creates a neurotoxic environment through the expulsion of reactive oxygen species and pro-inflammatory cytokines. The inflammation caused by the action of the microglia, in turn,

creates an environment in which brain tissues become critically hypoxic, disrupting mitochondrial function through inhibition of the electron transport chain, leading to premature cellular apoptosis[20].

To address the injury cascade and chronically neurotoxic environment created by the downstream consequences of TBI, we propose a treatment protocol designed to address these issues in sequence. The treatment protocol combines ketamine infusion therapy, hyperbaric oxygen therapy, nutrient-enriched intranasal platelet-rich plasma, and intranasal insulin delivered over the course of 90 days. The treatment methodology of this experimental protocol is discussed within this paper in hopes that clinical research can be designed to test the validity of this theory.

TBI Treatment Protocol Theoretical Framework

Problem

Traumatic Brain Injuries (TBIs) are a leading cause of death and disability in the United States. The Centers for Disease Control and Prevention (CDC) has estimated that annually, ~1.5 million Americans survive a traumatic brain injury [8]. The impact of TBI on society is immense, with TBI related healthcare expenditures eclipsing $48.3 billion each year [8]. However, the true burden of TBI is carried by the long-term complications suffered by its survivors and their loved ones; who face progressive cognitive, motor, and behavioral degradation due to neurodegenerative processes. TBI patients are also at a much higher risk of developing co-morbid depression. The degradation of neurological and psychosocial function is evidenced by the extremely high rate of unemployment for those who have suffered a TBI. A 2015 study on the unemployment rate in post-TBI working-age individuals found that 60.4% of TBI patients were unemployed two years following their initial injury [5].

Theory of TBI

A traumatic brain injury is defined as a nondegenerative, noncongenital insult to the brain from an external mechanical force, possibly leading to permanent or temporary impairment of cognitive, physical, and psychosocial functions, with an associated diminished or altered state of consciousness. TBIs are characterized by both primary acute damage to the brain from mechanical forces as well as delayed secondary damage triggered by biochemical mechanisms that evolve over time. The complex and interconnected nature of TBI must be understood to create a treatment protocol that addresses its mechanisms.

Acute Primary Injury

In a TBI the primary injury occurs as a result of the initial insult and persists, which causes displacement or deformation of the physical structures of the brain. Primary injuries to the brain can be broadly defined as focal and defuse, although they commonly occur in conjunction with one another. Focal injuries occur due to physical contact with the skull and may produce cerebral contusions and hematomas. Defuse injuries are caused by rotational inertial forces exerted on the brain, during a car accident for instance, which induce dynamic shear, compressive and tensile strains within the axons of the white matter tracts. The extent of axonal injury is a product of the

magnitude of strain and the rate of strain experienced by the brain [5]. Acute damage occurs instantaneously at the time of insult and leads to a cascade of inflammatory processes the first of which is the swelling of the axon at the injury site. This focal swelling of the axon continues following the injury as the normal delivery of substances through axonal transport continues. The swelling of the axon eventually leads to the collapse and detachment of the axon at the focal point of inflammation [18]. Similar damage is also done to the vasculature of the brain during the initial injury which plays a key role in the progression of secondary bio-chemical injuries.

Secondary Bio-Chemical Injury

TBI secondary injury includes a complex cascade of biochemical events involving excitotoxicity, mitochondrial dysfunction, oxidative stress, and neuroinflammation, leading to premature neuronal cell apoptosis. Damage to the vasculature of the brain during initial insult can cause hemorrhaging, edema, blood flow abnormalities, and blood-brain barrier disruption. The downstream consequences of this initial damage include hypoperfusion, altered delivery of metabolic substrates, and hypoxic and ischemic tissue damage[27]. Damage to the vasculature of the brain directly inhibits the delivery of necessary resources integral to the healing process, creating ischemia like environment. Since oxygen is the final electron acceptor in oxidative phosphorylation step IV, the creation of ATP, cellular mitochondria are unable to produce ATP through cellular respiration. A lack of oxygen also leads to an accumulation of lactic acid, through the process of anaerobic glycolysis. Lactic acid is an insufficient energy source to maintain cellular energy states. Intracellular ATP stores quickly deplete leading to a failure of cellular membrane ion pumps. Terminal membrane depolarization quickly follows which opens voltage-dependent Na+ and Ca2+ channels, the influx of which creates a catabolic state within the cell [33]. Terminal depolarization also causes the release of the excitatory neurotransmitter's glutamate and aspartate. Robust experimental evidence has linked the build-up of extracellular glutamate to excitotoxicity directly following a TBI [3,15]. The brain's reaction to both primary and secondary injuries is the deployment of microglial cells.

Microglia

Microglia are a native type of glial cell located throughout the central nervous system. Microglial cells contribute to the detection of and the response to changes in the central nervous system's physiological and pathological condition by altering their morphology, phenotype, and function[15]. In their normal resting state, microglial cells act as sentinel cells, continuously monitoring their environment for threats. If homeostasis in the brain is threatened by an imbalance or foreign cells, microglia activate altering their shape and function to take on a phagocytic role. In this activated state microglia produce and expel cytotoxic reactive oxygen species (ROS) and inflammatory cytokines including Interleukin-1β (IL-1β), Tumor necrosis factor-alpha (TNF-α), and Interleukin-6 (IL-6). Microglia produce and excrete of these chemicals to eliminate foreign cells found in the CNS, and then consume them through phagocytosis. In normal conditions, this process functions to clean and maintain the internal environment of the CNS. However, in the case of more severe TBI, when the system cannot fully repair itself, microglia become chronically activated creating a neurotoxic environment. This chronic

departure from homeostasis "primes" microglia to become more sensitive to subsequent insults to the brain [20].

Microglial Priming and Neuritic Beading

Chronically activated microglia exist in a state of constant arousal, expelling pro-inflammatory cytokines and ROS into the intercellular space. This state is highly persistent. In brain injury studies utilizing positron emission tomography (PET) microglial priming has been observed up to 17 years following initial injury This priming effect alters the ability of microglia to resolve the initial inflammatory process precipitated by the primary physical injury to the brain. We theorize based on previous research that the microglia's inability to resolve the initial inflammatory process is at least in part due to the hypoxic environment created by initial neuroinflammation. As stated above a hypoxic environment interrupts the process of ATP creation through cellular respiration. Therefore, it is likely that a primary cause of chronic microglial activation is mitochondrial dysfunction created by a lack of oxygen [20]. The hypoxic, neurotoxic environment also contributes to neuritic beading, the focal bead-like swelling of dendrites and axons, which is thought to be an early sign of neurodegeneration [28]. The cascade of TBI related primary and secondary physiological damage to the brain seems to translate directly into a variety of psycho-social comorbidities, in particular major depressive disorder.

Comorbid Depression

Although the exact nature of the relationship between physiological damage to the brain and the occurrence of psychological disorders requires further study, the increased risk of developing major depressive disorder following a TBI is well documented. The frequency of depressive disorders in TBI patients has been reported to range between 16-60%. The frequency of major depressive disorder in TBI patients is particularly noteworthy ranging from 24% to 34% of TBI patients (CITE). Fatigue (29%), distractibility (28%), anger or irritability (28%), and rumination (25%) were the most commonly reported depressive symptoms experienced by patients (CITE). We hypothesize that depression symptoms further exacerbate the inflammatory cascade present in TBI patients and play critical role in the inhibition of healing. Therefore, any potential treatment protocol for TBI must take into consideration the relief of depression and other comorbidities.

Additive Treatment Protocol

To address the multiplicity and complexity of both primary and secondary TBI injuries an additive protocol has been created that systematically targets the underlying causes of cellular damage and dysfunction. The goal of this protocol is to first return the brain to a state of pre injury homeostasis and then administer treatments that upregulate the body's natural healing processes.

1. Ketamine Infusion Therapy
    a. Relieving potential co-morbid depression symptoms
    b. Reducing levels of intracellular ROS

c. Down regulating the production of inflammatory cytokines
d. Upregulates Brain Derived Neurotropic Factor

2. Hyperbaric Oxygen Therapy
    a. Increases oxygen saturation of blood plasma
    b. Decreases inflammation
    c. Increasing cerebral blood flow
3. Intranasal Platelet Rich Plasma Infusions
    a. Delivers growth factors directly to the brain
4. Intranasal Insulin
    a. Increasing cellular glucose uptake
    b. Upregulates cellular respiration

Ketamine Infusion Therapy

TBI patients often suffer through the secondary phases of their injuries for years, enduring worsening symptoms and gradual cognitive and behavioral decline until finally seeking help. Often living with chronic pain and inflammation, these patients are also at high risk of developing comorbid depression. Ketamine hydrochloride is a non-selective N-Methyl-d aspartate (NMDA) glutamate receptor antagonist that elicits a dissociative psychedelic response in the brain. IV Ketamine is utilized as the first treatment in the protocol to provide relief from depression or symptoms, downregulate inflammation, and upregulate BDNF expression.

Antidepressant effects of Ketamine

Major depression is widely considered to be a common comorbidity with TBI. In a meta-analysis of the prevalence of comorbid TBI and depression, researchers found that up to 61% of TBI patients suffer from depression [17]. Ketamine has been proven clinically to produce rapid, profound, and surprisingly durable antidepressant effects [16]. The longevity of ketamine's antidepressant effect also seems to be positively correlated with the number and frequency of ketamine infusions [25]. To produce the greatest patient response to ketamine, the TBI treatment protocol utilizes an increasing dosage schedule of ketamine administered intravenously over four infusions. Dosing begins at .5mg/kg and increases, based on patient tolerance, by .2mg/kg each subsequent infusion up to a maximum dose of 1.1mg/kg at the fourth infusion. The methodology for this gradually increased dosing schedule is based on research performed with other compounds, such as psilocybin, that suggests that the anti-depressant effects of psychedelic compounds are directly related to the novel experience that patients undergo while under the influence of the drug. The relief of depression symptoms can also be explained through the biochemical effects of the drug on the nervous system.

Ketamine and BDNF Upregulation

BDNF is a vital neurotrophin that exhibits high levels of expression in mammalian brains. BDNF is responsible for the regulation of synaptic repair, protection, and growth, even in the presence of various neurotoxins and ROS. The idea that degenerative diseases of the nervous system may result from an insufficient supply of neurotrophic factors has generated great interest in BDNF as a potential therapeutic agent. Many reports have documented evidence of decreased expression of BDNF in neurological disease [22]. The mechanism of ketamine related BDNF release is detailed in figure 1. This figure illustrates the emerging theory behind the antidepressant effects of ketamine. These effects are initiated by ketamine's blockade of postsynaptic, GluN2B-containing NMDA receptors. When overstimulated, these receptors activate eukaryotic elongation factor-2 (eEF2) and depress BDNF levels. The blockade of these NMDA receptors triggers the shuttling of α-amino-3- hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptors to the synapse, enhancing synaptic efficacy. The activation of AMPA receptors begins a signaling cascade that raises BDNF levels, which counters the down-regulation caused by eEF2, and releases BDNF into the intracellular space, the effects of which will be discussed in the following section. Local release of BDNF is also thought to stimulate tropomyosin receptor kinase B (TrkB), engaging relevant signaling cascades and resulting in the activation of the molecular target of rapamycincomplex1(mTORC1). This step, in turn, activates local protein synthesis necessary for increasing dendritic spine formation and restoring synaptic connectivity, in a process known as neuro-regeneration. Ketamine also may generate its antidepressant effects indirectly by blocking NMDA receptors on GABA interneurons. In this way, ketamine reduces the release of glutamate [17].

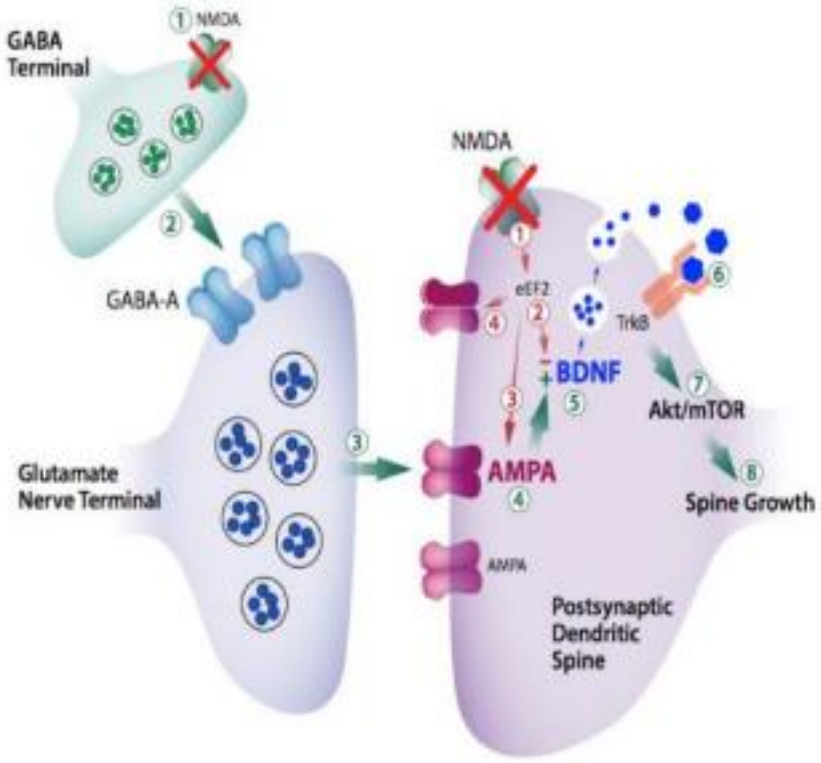


Figure 1: Glutamate mediated BDNF Upregulation

Ketamine's Role in Inflammation Reduction

Chronic inflammation plays a key role in the secondary injuries experienced by TBI patients. The immune response of macrophage cells to trauma is the release of pro-inflammatory cytokines, nitric oxide, and oxidative substance into the point of injury. Under normal conditions, the resulting upregulation of inflammation at the point of injury would signal the process of cellular healing to begin. However, in the case of microglial priming, microglial cells enter a state of prolonged activation in which they continually release proinflammatory cytokines

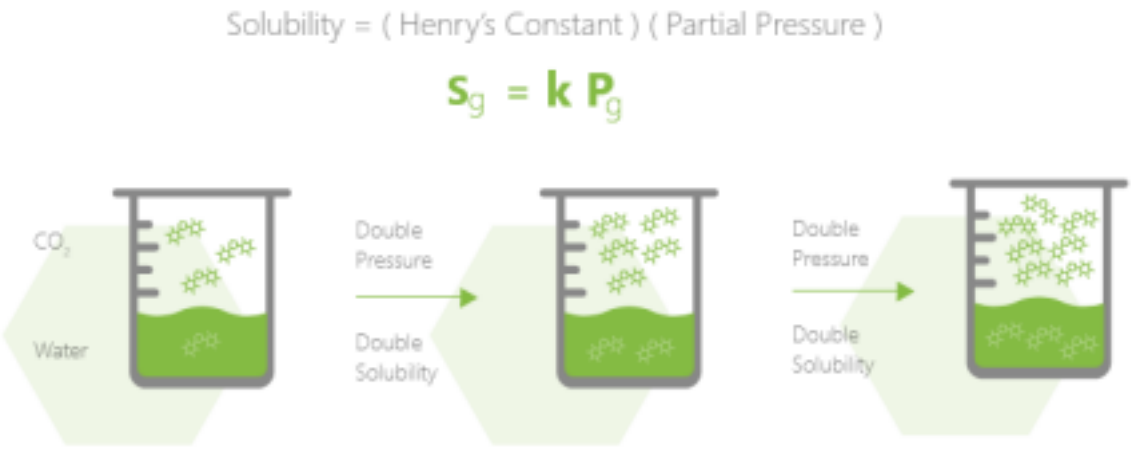
[15]. The effects of ketamine on these functions have been described in several in vitro studies. Ketamine inhibits cytokine production (Interleukin 6 and Tumor necrosis factor-alpha) and nitric oxide production in preparations of isolated and immune-stimulated macrophages [1]. Moreover, the oxidative function of the microglial cells is also inhibited by ketamine, making it an ideal therapeutic agent for the treatment of TBI[1]. Following the administration of ketamine, patients begin a regimen of 40 hyperbaric oxygen treatments.

Hyperbaric Oxygen Therapy

The brain is a highly aerobic organ. The brain receives ~15% of cardiac output, consumes ~20% of bioavailable oxygen, and utilizes ~25% of the total body glucose. At a standard healthy condition, at any given time, the brain is utilizing almost all oxygen/energy delivered to it. The regeneration process following a TBI requires a substantial amount of additional energy. The increased oxygen levels in the blood and body tissues produced by hyperbaric oxygen treatment (HBOT) can supply the energy needed for brain repair [26].

Hyperbaric Oxygen Therapy involves exposing the body to 100% oxygen while under greater than normal atmospheric pressures. HBOT harnesses the principle of Henry's Law to heal wounds. The patient enters the hyperbaric chamber and pressure is increased inside the chamber to between 1.5 - 3 atmospheres absolute (ATA), approximately 1.5 - 3x normal atmospheric pressure at sea level. Once at pressure, medical-grade oxygen is pumped into the chamber. The

Figure 2: Henry's law



patient remains in the chamber breathing in concentrated oxygen for 60-90 minutes each treatment. The exposure to oxygen at pressure has profound effects on the body.

At sea level (1 ATA, 14.7psi) blood plasma oxygen concentration averages .3ml per deciliter. Tissues at rest extract 5 to 6 ml of oxygen per deciliter of blood, assuming normal perfusion. Administering 100 percent oxygen at ambient pressure increases the amount of oxygen dissolved in the blood fivefold to 1.5 ml per deciliter, and at 3 atmospheres, the dissolved-oxygen content is approximately 6 ml per deciliter, more than enough to meet resting cellular requirements without any contribution from oxygen bound to hemoglobin [32]. Since the oxygen is dissolved into the plasma solution it can reach obstructed areas that red blood cells are unable to pass through. The hyperoxygenation resulting from HBOT also stimulates immune function by upregulating white blood cell creation, enhances cellular phagocytic capabilities, and accelerates neovascularization in hypoxic areas [26].

HBOT and TBI Treatment

The primary mechanism of injury in a TBI is the diffuse shearing of axonal pathways and small blood vessels, also known as diffuse axonal injury. The follow-on secondary injuries caused by microglial priming; reactive oxygen species further impair the brain's ability to heal by creating inflammation-related tissue hypoxia. Global brain hypoperfusion, and its related tissue ischemia, detected in patients suffering from TBI, serves as a rate-limiting factor for any regenerative process [26]. By increasing the oxygen level in blood and body tissues, HBOT can augment the body's natural repair mechanisms. The successful clinical administration of HBOT for TBI is

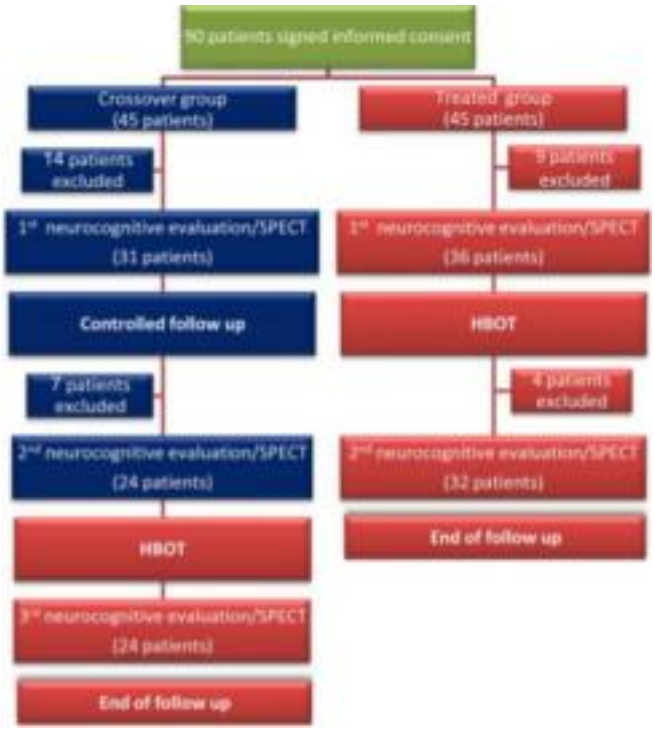


Figure 3: HBOT Study Design

documented in the work of Rahav Boussi-Gross and colleges at the Institute of Hyperbaric Medicine, Assaf Harofeh Medical Center, Zerifin, Israel. The crossover-controlled study was conducted with 90 participants aged 18 years or older who had suffered mild TBI (less than 30 minutes loss of consciousness) 1-6 years prior to the study and continued to suffer from post concussive symptoms. The study used a combination of Single Positron Emitted Topography (SPECT) imaging, cognitive testing, and quality of life assessments to measure the effectiveness of 40 sessions of HBOT. Cognitive functions were assessed across four indices associated with four fundamental higher brain functions informational processing speed, attention, memory, and executive function. Quality of life was assessed using the EQ-5D questionnaire. The EQ-5D is a preference-based health-related quality of life (HRQL) questionnaire that generically evaluates the quality of life, based on answers in the five-question categories of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.

Patients in the treated group were evaluated using neurocognitive testing and SPECT imaging twice, at baseline, and after 40 treatments of HBOT. Patients in the crossover group were evaluated three times, at baseline, after a two-month control period of no treatment, and after 40 treatments of HBOT. The post-HBOT neurological evaluations, as well as the SPECT scans, were performed between one to three weeks after the end of the HBOT protocol. The following HBOT protocol was administered: 40 daily sessions, 5 days/week, 60 minutes each, 100% oxygen at 1.5ATA [26].

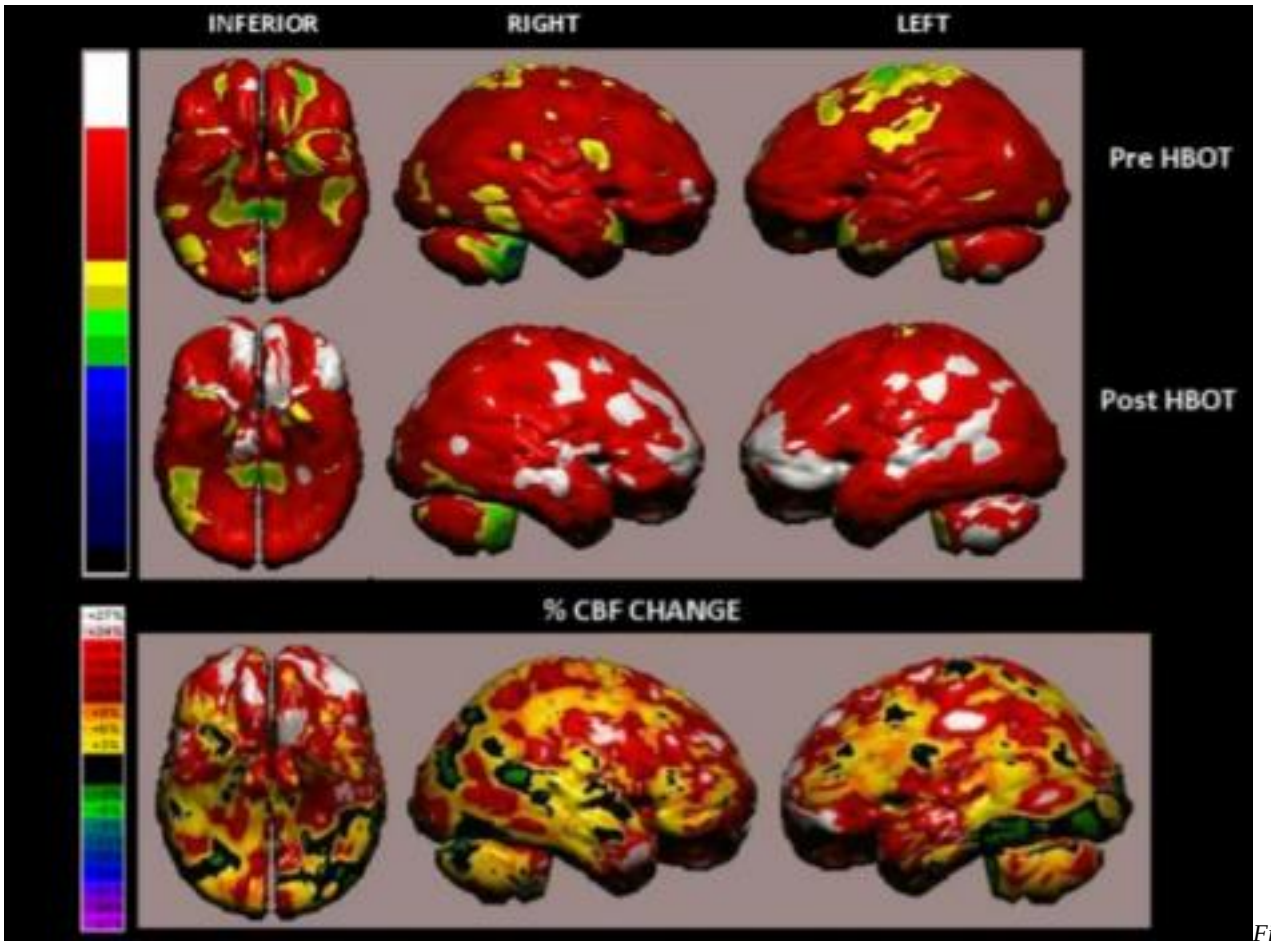


Figure 4: Changes in CBF Following 40 HBOT Treatments

The study found that TBI patients responded positively to HBOT treatment. The SPECT imaging produced profusion values for each Brodmann Area (BA) in the brain. The results revealed a significant increase in brain perfusion following 40 HBOT treatments. A summary of the results is presented in figure 4. To construct the figure we calculated, for each patient, the relative change in the SPECT measured brain activity during each phase of the trial. "The relative change was defined as the difference in normalized activity between the endpoint and the start point divided by the activity at the start. We calculated, for each BA, the average changes overall patients that underwent HBOT (both treated and cross groups). These results correspond to the red curve. The blue curve represents the results of similar calculations for the control period (averaging for each BA over all the results of all patients in the crossover group during the control period)" [26]. The results revealed significant improvement in brain perfusion following HBOT in the frontal and temporal areas, including the inferior frontal gyrus (BA 45), the middle frontal area (BA 46), parts of the orbitofrontal cortex (BA 47 and 11), most of the temporal cortex (BA 36, 37, 38, 20, 21, 22, 28), and parts of the Cingulate gyrus (BA 24, 25)[26]. This data can be better visualized by viewing the SPECT image of one of the study participants. The SPECT image below is from a 51-year old woman in the treatment group who suffered an mTBI 2 years before the study. Baseline perfusion values are shown on the top row while post treatment

perfusion values are shown in the middle row the change in cerebral blood flow (CBF) is shown in the bottom row.

The effect of HBOT on cognitive function mirrored the results of the SPECT scans. The mean of the relative change, compared to the baseline testing cognitive scores, was calculated and graphed. Figure 5 shows the mean relative changes in all four cognitive indices for the crossover

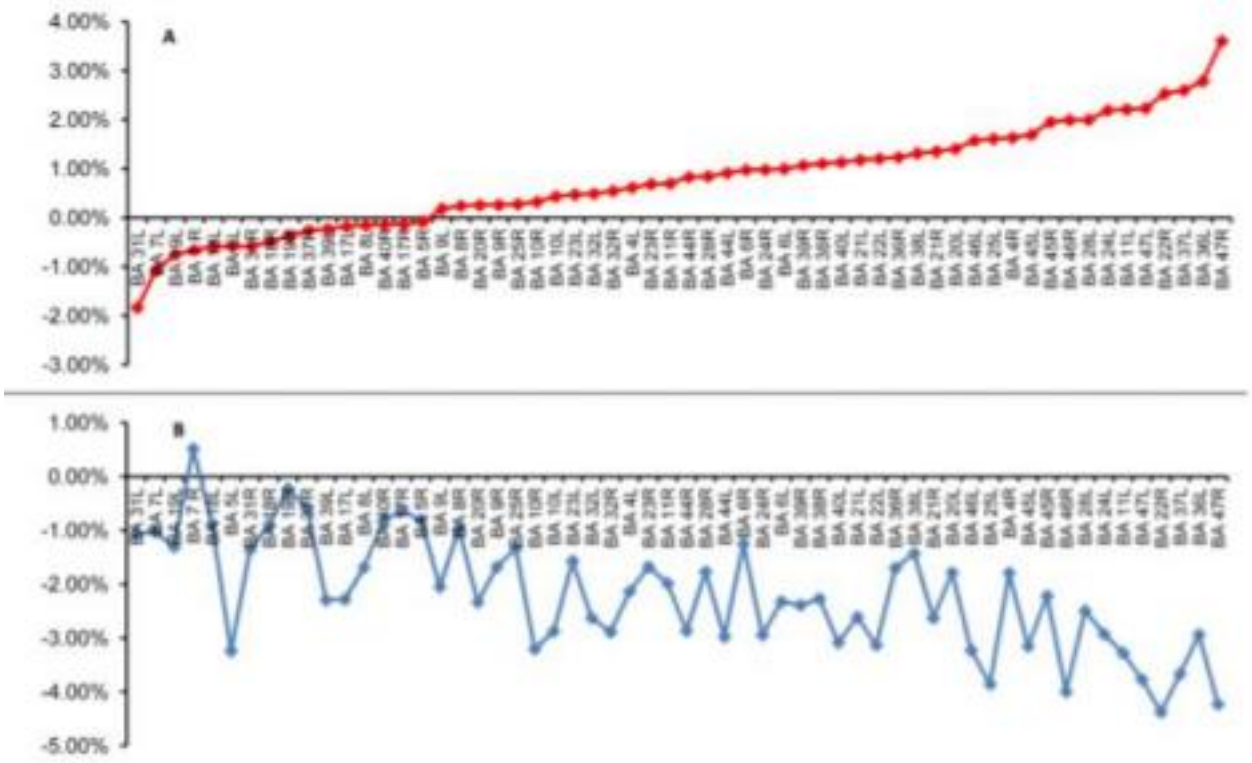


Figure 5: Brain Perfusion Scores Following 40 HBOT Treatments

group following the control period and following HBOT, and for the treated group following HBOT. A significant improvement was observed in both the treatment and crossover groups in every cognitive measure.

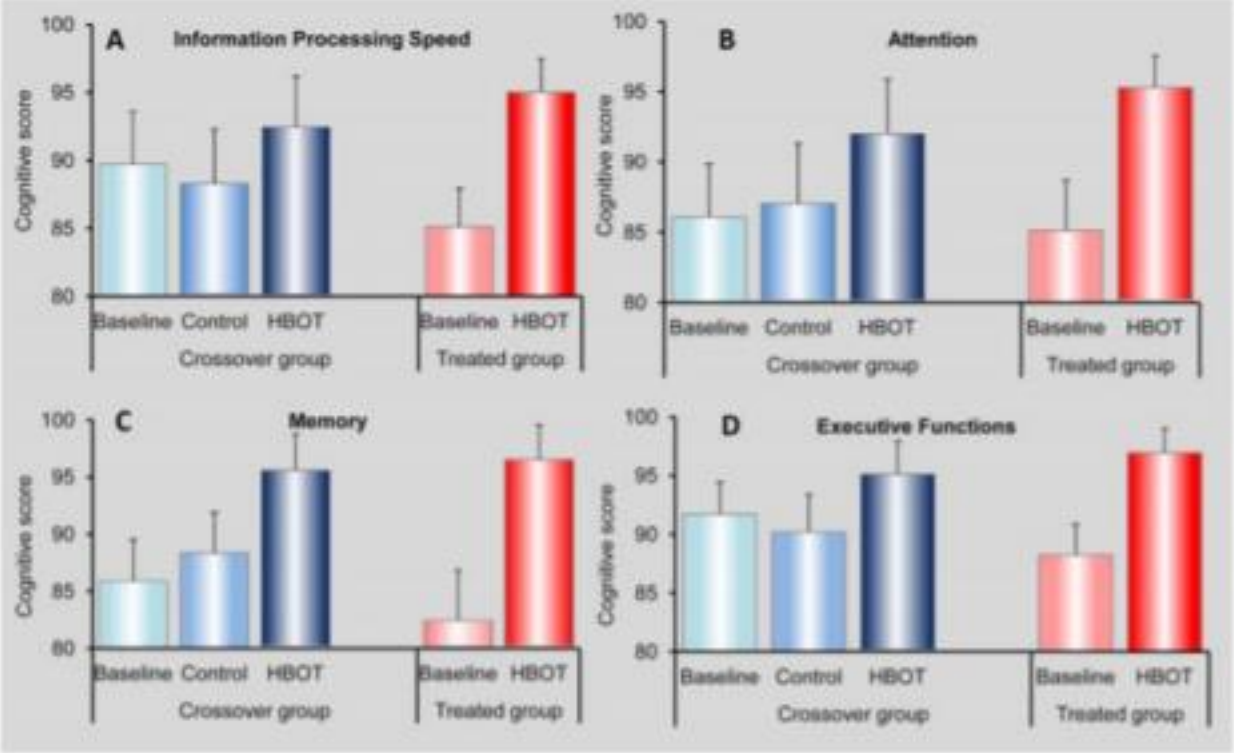


Figure 6: Cognitive Function Scores Following 40 HBOT Treatments

Antioxidant Effects of HBOT

A concern regarding the delivery of additional oxygen into the body following a TBI is the creation of additional ROS in the body. Recent research has demonstrated that HBOT dramatically increases plasma oxygen saturation it also induces an upregulation in the body's antioxidant defenses. Experiments with invitro endothelial cells, the cells targeted for wound healing in HBOT therapies, found that HBOT activated the genetic expression of protective genes. Genes uncovered in this experiment include the 70-kilodalton heat shock protein (HSPA1A), heme oxygenase 1 (HMOX1), and metallothionein 1X (MT1X), which collectively can provide protection from metabolic, proteotoxic, and oxidative forms of stress [9]. In the case of TBI, where ROS run rampant within hypoxic tissues, these effects provide protection from premature cellular apoptosis.

HBOT Upregulates Stem Cell Production

One of the most powerful second-order effects that HBOT has on the body is enhancing the creation of pluripotent bone marrow-derived stem/progenitor cells (SPCs). Pluripotent SPCs exhibit properties similar to embryonic stem cells and can differentiate into many different kinds of cells. After one HBOT treatment at 2.0 ATA for 2 hours the population of cells expressing the CD34+ stem cell marker doubled. Over the course of 20 HBOT treatments circulating 34D+ expressing cells increased eightfold [31]. Further investigation into the relationship between the partial pressure of oxygen and the mobilization of SPC's has revealed that a correlation exists. Blood from twenty patients was obtained before and after the 1st, 10th and 20th HBOT treatment

using protocols involving exposures to oxygen at either 2.0 or 2.5 ATA. Post-treatment values of CD34+, CD45-dim leukocytes were always 2-fold greater than the pre-treatment values for both protocols. Values for those treated at 2.5 ATA were significantly greater than those treated at 2.0 ATA by factors of 1.9 to 3-fold after the 10th and before and after the 20th treatments [11].

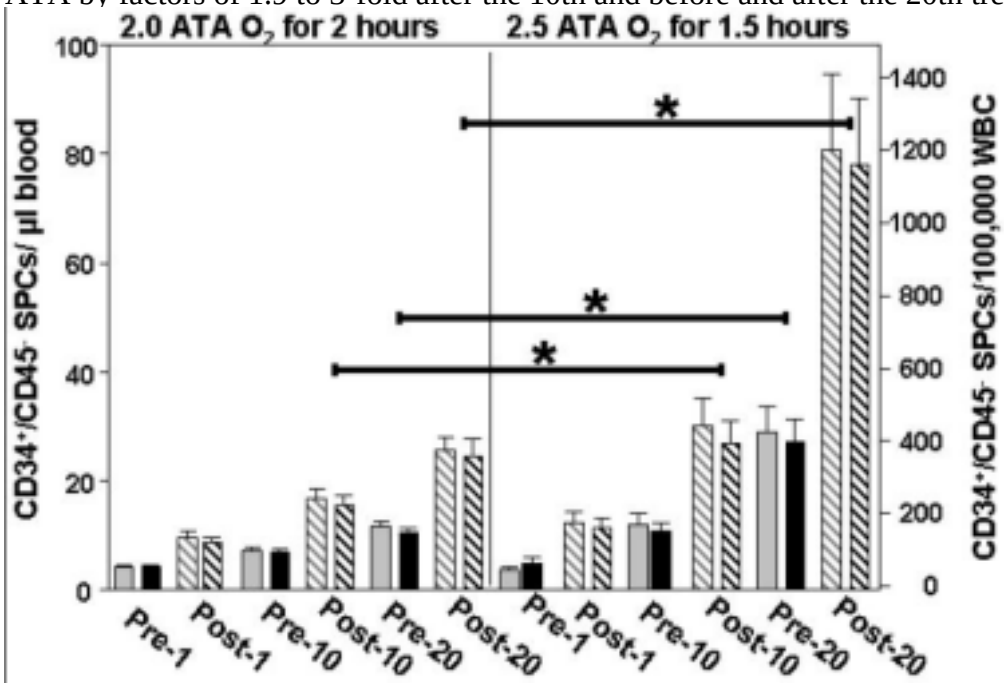


Figure 7: Upregulation of SPC's following HBOT

The relationship between partial pressure and stem cell mobilization can be utilized therapeutically to treat TBI. Stem cells can be easily harvested along with hyper oxygenated plasma, concentrated and delivered to areas of the body or the brain that have been damaged by acute injury.

Intranasal Platelet Rich Plasma

Platelet-rich plasma (PRP) is an autologous concentration of human platelets derived from whole blood. Concentrations of human platelets are rich in the fundamental protein growth factors excreted by platelets to facilitate wound healing. These growth factors include the 3 isomeres of platelet-derived growth factor (PDGF), two of the numerous transforming growth factors (TGF1 and TGF2), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), insulin like growth factor (IGF), fibroblast growth factor (FGF) [21,24]. The primary growth factors present in PRP and their functions are listed in (Figure 8.) Growth factors derived from PRP can contribute to tissue regeneration, by assisting cell migration, proliferation, differentiation, and extracellular matrix synthesis [21]. The use of PRP to accelerate healing following surgical procedures is well documented, due to its autologous nature is also stands to reason that PRP could be a viable treatment for TBI if administered intranasally.

Name	Abbreviation	Function
Platelet derived growth factor	PDGF	Enhances collagen synthesis, proliferation of bone cells, fibroblast chemotaxis and proliferative activity, macrophage activation
Transforming growth factor β	TGF-β	Enhances synthesis of type I collagen, promotes angiogenesis, stimulates chemotaxis of immune cells, inhibits osteoclast formation and bone resorption
Vascular endothelial growth factor	VEGF	Stimulates angiogenesis, migration and mitosis of endothelial cells, increases permeability of the vessels, stimulates chemotaxis of macrophages and neutrophils
Epidermal growth factor	EGF	Stimulates cellular proliferation, differentiation of epithelial cells, promotes cytokine secretion by mesenchymal and epithelial cells
Insulin-like growth factor	IGF	Promotes cell growth, differentiation, recruitment in bone, blood vessel, skin and other tissues, stimulates collagen synthesis together with PDGF
Fibroblast growth factor	FGF	Promotes proliferation of mesenchymal cells, chondrocytes and osteoblasts, stimulates the growth and differentiation of chondrocytes and osteoblasts

Figure 8: Growth Factors Present in PRP

Intranasal Administration Efficacy

Intranasal delivery provides an expedient non-invasive method to deliver therapeutic agents to the brain. The blood-brain barrier (BBB) limits numerous therapeutic agents from entering the central nervous system (CNS) based on the molecular size or charge. Through intranasal administration therapeutic agents can bypass the BBB, ensuring delivery directly to the CNS in minutes. This is possible because of the unique connections that the olfactory and trigeminal nerves provide between the brain and the external environment. Intranasally administered therapeutics reach the CNS via the olfactory and trigeminal neural pathways. Both the olfactory and trigeminal nerves innervate the nasal cavity, providing a direct connection with the CNS [10,12]. We utilize this method of administration to deliver a cocktail containing PRP and other vital nutrients directly to the brain to treat TBI.

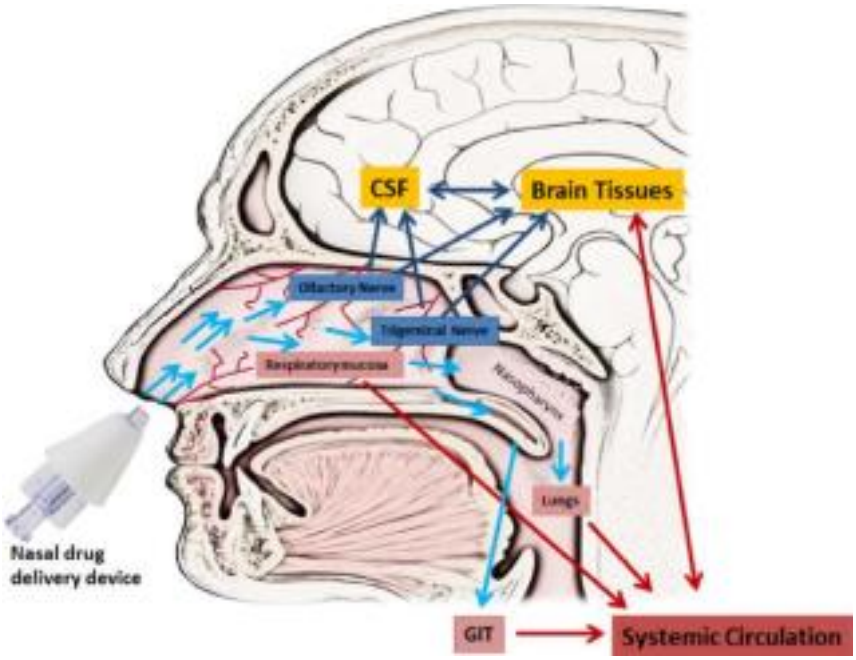


Figure 9: Intranasal Delivery

PRP+

To maximize the effectiveness of intranasally administered PRP the plasma concentration is mixed with glutathione and nicotinamide adenine dinucleotide+(NAD+). Glutathione is a

compound integral to cellular detoxification and is involved in the excretion of oxidative toxins from the body. Glutathione directly scavenges diverse oxidants: superoxide anion, hydroxyl radical, nitric oxide, and carbon radicals. Glutathione catalytically detoxifies hydroperoxides, peroxynitrites, and lipid peroxides. Another way glutathione protects cells from oxidants is through the recycling of vitamins C and E. Low levels of glutathione are associated with not only chronic exposure to chemical toxins but neurodegenerative disorders. To better facilitate the healing processes provided by PRP's inherent growth factors glutathione is delivered directly to the brain to neutralize ROS and other neurotoxins, paving the way for the regenerative effects for HBOT and PRP to take hold [23]. By further combining the intranasally delivered PRP and Glutathione with nicotinamide adenine dinucleotide (NAD+) cellular metabolism can be further upregulated and cellular apoptosis can be averted.

NAD+ is a coenzyme found in all living cells. Substantial evidence has indicated that NAD+ plays a critical role in both cellular metabolism and apoptosis. Excessive poly (ADP-ribose) polymerase-1(PARP-1) activation plays a significant role in ischemic brain damage. Increasing evidence has supported the hypothesis that PARP-1 induces cell death by depleting intracellular NAD+[34]. By providing the cells of the brain an exogenous source of NAD+ we hope to upregulate cellular metabolism and prevent further cellular apoptosis.

Intranasal Insulin

Cellular healing is a highly energy-intensive process. The compounded effects of neural inflammation and toxicity create an environment within the brain that is not conducive to healing. To facilitate the healing process additional energy must be introduced into the system. Following an initial twenty HBOT treatments patients will begin a regimen of intranasal insulin treatments.

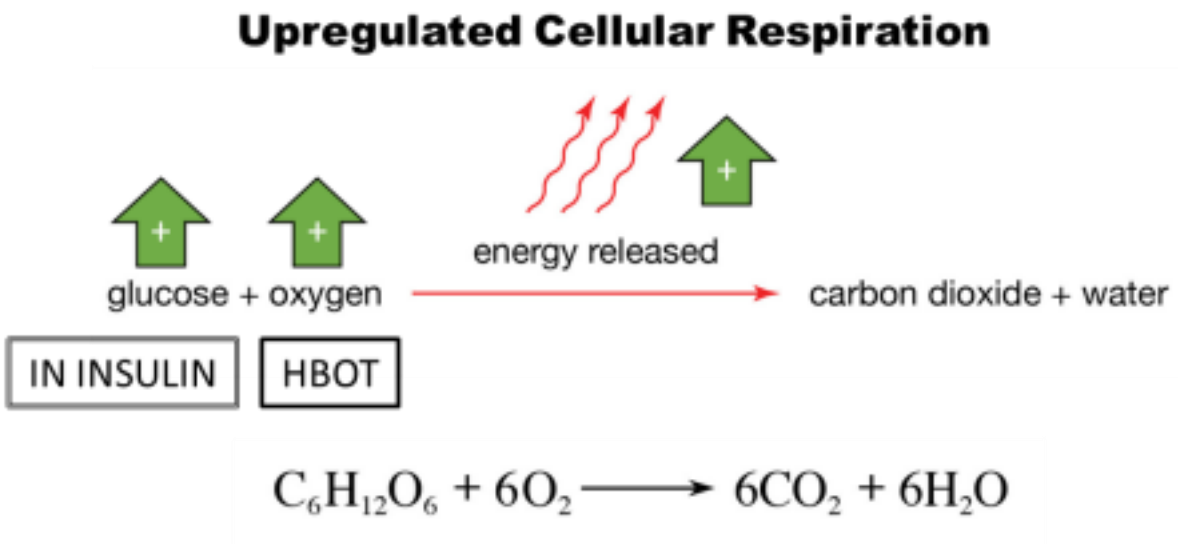


Figure 10: Upregulated Cellular Respiration

The ability of cells to utilize glucose for the creation of ATP through cellular respiration is regulated in part by insulin levels. Over the past decade, several studies have demonstrated an association between Alzheimer's Disease (AD) and decreased insulin signaling [13]. It has become increasingly evident that oligomeric aggregates of both amyloid-beta (Aβ) and tau contribute to this synaptic dysfunction that precedes the cognitive decline seen in AD. Notably, decreased insulin receptor function increases synaptic sensitivity to the binding of and dysfunction caused by Aβ, and insulin and insulin-sensitizing therapy have been shown to be effective for cognition in mouse models of AD as well as in patients with mild cognitive impairment (MCI) or early AD. Furthermore, AD patients exhibit insulin resistance and decreased insulin signaling response in the hippocampus [7].

The inclusion of intranasal insulin into the treatment protocol is designed to create an additional synergistic effect with HBOT through the upregulation of cellular respiration. By simultaneously increasing the bioavailability of glucose through intranasal insulin and oxygen saturation through HBOT greater amounts of ATP can be produced and utilized to facilitate healing. Insulin is administered intranasally rather than intravenously to prevent the exacerbation of peripheral insulin resistance. Intranasal delivery provides a method for rapid delivery of insulin to the CNS along olfactory and trigeminal perivascular channels without adversely affecting blood insulin or glucose levels [4].

Conclusion

Traumatic brain injuries create a complex cascade of effects throughout the CNS that can lead to permanent damage and dysfunction of the brain. We have created a treatment protocol that targets the underlying mechanisms of the TBI process. Through the administration of ketamine infusions, hyperbaric oxygen, intranasal PRP and intranasal insulin treatments we theorize that

cellular damage to the brain can be repaired.

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