Hyperbaric Oxygen Therapy in the Treatment of Acute Severe Traumatic Brain Injury: a Systematic Review

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ABSTRACT

There has been no major advancement in a quarter of a century for the treatment of acute severe traumatic brain injury (TBI). This review summarizes 40 years of clinical and pre-clinical research on the treatment of acute TBI with hyperbaric oxygen therapy (HBO_2) in the context of an impending National Institute of Neurologic Disorders and Stroke (NINDS)-funded, multicenter, randomized, adaptive Phase II clinical trial – the Hyperbaric Oxygen Brain Injury Treatment (HOBIT) trial. Thirty studies (8 clinical and 22 pre-clinical) that administered HBO_2 within 30 days of a TBI were identified from PubMed searches. The pre-clinical studies consistently reported positive treatment effects across a variety of outcome measures with almost no safety concerns, thus providing strong proof-of-concept evidence for treating severe TBI in the acute setting. Of the 8 clinical studies reviewed, 4 were based on the senior author's (GR) investigation of HBO_2 as a treatment for acute severe TBI. These studies provided evidence that HBO_2 significantly improves physiologic measures without causing cerebral or pulmonary toxicity and can potentially improve clinical outcome. These results were consistent across the other 4 reviewed clinical studies, thus providing preliminary clinical data supporting the HOBIT trial. This comprehensive review demonstrates that HBO_2 has the potential to be the first significant treatment in the acute phase of severe TBI.

Keywords: Hyperbaric Oxygen; Traumatic Brain Injury; Normobaric Hyperoxia; Glasgow Coma Scale; Glasgow Outcome Scale

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INTRODUCTION

Traumatic brain injury (TBI) has enormous negative social and economic impacts across a large variety of populations. Nearly 4 million people in the United States suffer a TBI each year – half of whom require a visit to the Emergency Department, 500,000 of whom are hospitalized, and 50,000 of whom die from their injury.[1] The risk of death and long-term disability to a patient rises considerably with increasing injury severity and concomitant body trauma. It is estimated that 2% of the US population – approximately 5.3 million people – are living with long-term disabilities related to their TBI.[2] The annual combined direct and indirect financial impact incurred by TBI in the United States is $76.5 billion.[3] Despite these physical and financial costs however, there has been little advancement in the acute treatment of TBI since the 1990's,[4] and clinical outcomes have not improved. In fact, in the last 15 years at least 25 clinical trials of therapeutics for TBI have failed.[5]

Many treatments administered in the immediate period following a TBI are focused on altering the acute pathophysiology. However, following the primary mechanical injury to the brain, secondary injury frequently develops. This secondary injury is precipitated by ischemia resulting from decreased cerebral blood flow (CBF) and is particularly likely to occur in the first 24 hours after injury.[6, 7] Because of decreased oxygen (O_2) delivery to brain cells,[8] the brain converts from aerobic to highly inefficient anaerobic metabolism, resulting in inadequate energy production in the brain and eventual cell death.

Hyperbaric oxygen therapy (HBO_2) targets TBI-induced ischemia by exposing patients to an environment that substantially increases the amount of O_2 inspiration (100% O_2 at >1 ATA), producing an increased O_2 concentration in the plasma and thus increased delivery of O_2 for diffusion to brain tissue. Despite the capacity of HBO_2 to protect against secondary ischemic damage, the use of HBO_2 for the treatment of TBI has been controversial. One concern regarding the use of HBO_2 for acute TBI arises from apparent conflicts in the literature regarding its efficacy. It is likely that injury heterogeneity, variable injury chronicity, and variability in study design have contributed to this perception. Additional concerns relate to O_2 toxicity and the logistics of widespread implementation of this therapy.

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Consideration of HBO_2 for the treatment of acute TBI is warranted, as evidenced by the fact that a multicenter study across 15 US academic centers was recently awarded National Institute of Neurological Disease and Stroke (NINDS) funding under the auspices of SIREN (Strategies to Innovative Emergency Care Clinical Trials Network).[9] The rigorously designed adaptive Phase II Hyperbaric Oxygen Brain Injury Treatment (HOBIT) trial will enroll 200 TBI patients with a specific subset of pathology to assess the efficacy of HBO_2.[9] In this review, we summarize the pre-clinical and clinical studies utilizing HBO_2 for the treatment of acute TBI conducted to date. We also discuss the neuroprotective mechanism of HBO_2 and its potential clinical utility to treat acute severe TBI, the controversy surrounding its use, and briefly, the methodology of the HOBIT tria.

MATERIALS AND METHODS

A PubMed literature search was performed on February 22, 2016 to identify primary articles on the acute use of HBO_2 or combined HBO_2 and normobaric hyperoxia (NBH; 100% O_2 at 1 ATA) for TBI in both the clinical and pre-clinical settings:

- "hyperbaric oxygenation"[MeSH Terms] AND "brain injuries"[MeSH Terms] AND (Clinical Trial[ptyp] AND "humans"[MeSH Terms]),
- "hyperbaric oxygenation"[MeSH Terms] AND "brain injuries"[MeSH Terms] AND "animals"[MeSH Terms:noexp],
- "brain injuries"[MeSH Terms] AND "normobaric hyperoxia"[All Fields] AND "humans"[MeSH Terms], and
- "brain injuries"[MeSH Terms] AND "normobaric hyperoxia"[All Fields] AND "animals"[MeSH Terms:noexp].

These PubMed searches revealed a total of 46 clinical and 77 pre-clinical studies. Studies that employed a treatment that combined HBO_2 with NBH were included. Studies were excluded if the total sample size of the treatment groups was less than 6 or an English translation was not readily available. Clinical studies were excluded if treatment was initiated >30 days post-injury and if participants with non-traumatic brain injuries (ie, stroke, hypoxia, etc) were enrolled, unless the authors included data on participants with isolated TBI. Pre-clinical studies were excluded if the treatment was given prior to the induced injury or if the induced brain injury did not model TBI (ie, ischemic, cortical stab

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injury, anoxic, cryogenic, etc). Studies included that were not found in the indicated searches were reviewed in an identical manner to papers obtained through PubMed.

RESULTS

Twenty-two pre-clinical studies (20 of which implemented HBO_2 and two of which implemented combined HBO_2 and NBH) and 7 clinical studies (6 that implemented HBO_2 and 1 that implemented combined HBO_2 and NBH treatment) met the inclusion criteria for this review.

Pre-clinical studies

HBO_2 treatment

Twenty pre-clinical studies utilizing a wide range of methodologies employed HBO_2 to acutely treat induced TBI. Adult male Sprague-Dawley rats were used in 15 (75%) studies, whereas the remaining 5 studies used rabbits (n = 2), Wistar rats (n = 2), or mice (n = 1). The TBI model most commonly used was cortical impact (CI; n = 8), but dynamic cortical deformation (DCD; n = 6), lateral fluid percussion (LFP; n = 5), and blast injury (n = 1) were also utilized. Treatment regimens included pressures between 1.5 and 3 ATA for 30 to 90 minutes, and all but 2 studies initiated treatment within 6 hours of the injury. Seven studies administered a single HBO_2 treatment, 2 studies administered 2 consecutive daily treatments, 7 studies administered at least 3 daily treatments, and 4 studies administered multiple treatments per day for at least 3 days.

Physiologic outcomes

The pre-clinical models provided evidence for the neuroprotective effect of HBO_2 after TBI, reporting reduced lesion size,[10-13] lesion severity,[14] brain water content,[10, 14-16] and apoptosis.[10, 14, 16-21] In fact, all 7 of the studies that assessed neural apoptosis reported decreased apoptosis in animals treated with HBO_2 after induced injury, as measured by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) cell staining. Further, these studies also reported reduced levels of apoptosis-related proteins (B-cell lymphoma 2 [Bcl-2]; B-cell lymphoma-extra-large [Bcl-xL]; bcl-2-associated X protein [Bax]; caspase-3; and caspase-9) in treated animals, providing further evidence of the neuroprotective effect of HBO_2. The transmembrane potential in mitochondria, measured

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by caspase-9 activity, was found to be significantly reduced after injury and was subsequently brought back to near-normal levels following HBO_2, thus reducing activation of the mitochondrial apoptotic pathway.[18, 19]

Apoptosis within the hippocampus and general hippocampal neuronal integrity has also been repeatedly shown to benefit from HBO_2, potentially through an anti-inflammatory mechanism.[12, 14, 15, 22] The inflammatory response of animals with an induced injury is consistently reduced after HBO_2 compared to both baseline measurements and those animals that do not receive treatment. This response has been shown through serum and cortex measurements of biomarkers, including neutrophil infiltration, tumor necrosis factor-alpha (TNF-α), transforming growth interacting factor (TGIF), transforming growth factor-beta1 (TGF-β1), interleukin-1beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), macrophage inhibitory protein-2 (MIP-2), monocyte chemotactic protein-1 (MCP-1), matrix metalloproteinase-9 (MMP-9), hypoxia inducible factor-alpha (HIF-α), and myeloperoxidase (MPO) activity.[10, 15, 16, 23-26] Those animals that displayed reduced inflammatory responses following HBO_2 had consistently better functional outcomes and reduced lesion volumes. Chen et al. improved the mechanistic understanding of the positive anti-inflammatory effect of HBO_2 when they reported that mice injected with an anti-inflammatory protein, IL-10, following CI had better functional outcomes, and that mice with an induced genetic anti-inflammatory defect (IL-10 knockout) had greater lesion volumes, elevated apoptosis, and worse functional outcomes than wild-type mice after CI.[10]

Additional support for the neuroprotective effect of HBO_2 after TBI include findings of reduced blood brain barrier (BBB) permeability and dysfunction[10, 15, 16, 27] and infarction volume,[23, 24, 26] as well as increased neuronal density, neuronal integrity, neurogenesis, synaptogenesis, and axonal integrity.[11, 16, 18, 24, 28] Only one study reported neutral treatment effects, but its sole outcome measure was cerebral edema.[29]

Functional and cognitive outcomes

In pre-clinical studies, HBO_2 was shown to have a positive effect on functional and cognitive outcomes. Treatment-dependent improvements were seen in overall motor function,[23, 26] cognitive and behavioral testing,[11, 24] neurologic function,[14, 27] and locomotor coordination,[28] as well as in specific tests such as the Morris water maze,[15, 22] grip-strength

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test,[28] and beam-walk test.[12] Wang et al. designed a study to determine the impact of the post-injury window (ie, the time between the injury and the initiation of treatment) and number of treatments on improvement in neurological function.[14] The authors reported that a single treatment initiated within 12 hours of injury led to improved neurologic outcomes compared with a longer window of 24 hours; no significant improvement was observed with a 72-hour window before a single treatment. However, if the first HBO_2 treatment was initiated at 24 hours post-injury, multiple HBO_2 treatments (either 3 or 5 consecutive days) were significantly more effective than a single treatment for decreasing both neurologic deficit scores and neuronal cell loss. Improvements were still seen if the first treatment was initiated within 48 hours of injury and followed by additional treatments, although these improvements were less robust than those observed in response to a single treatment administered at 6 hours. This data suggests that the optimal treatment paradigm for clinical studies may be a single treatment initiated within 24 hours of the injury followed by treatments for 5 consecutive days.

Safety

Of the 20 studies reviewed, only 1 suggested a negative effect of HBO_2 treatment. Tinianow et al. reported that 4 animals died from O_2 toxicity during their study, and some other animals temporarily lost motor function in the forepaw.[12] The authors of this study initiated treatments with a 145-minute dive that reached 2.5 ATA. This is an exceptionally high dose that would have caused the formation of reactive oxygen species (ROS) across many organ systems, including the central nervous system, to levels that easily exceeded the body's antioxidant mechanisms, resulting in large-scale, unrepairable cellular damage (ie. lipid peroxidation and DNA destruction) and inevitable fatality.

Combined HBO_2 and NBH treatment

Two pre-clinical studies combined HBO_2 and NBH into 1 treatment, both of which used a LFP model of TBI in adult male Sprague-Dawley rats.[30, 31] Treatment was initiated 15 or 30 minutes after the injury. Zhou et al. implemented HBO_2 (1.5 ATA) for 1 hour prior to 3 hours of NBH.[30] Daugherty et al. used the same methodology in 1 group of rats, and NBH for 30 minutes prior to HBO_2 (1.5 ATA) on a second group. All animals in both studies were exposed to 1 treatment before sacrifice.

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Physiologic outcomes

The brain tissue oxygen tension ($P_{bt}O_2$) of animals treated with NBH prior to HBO$_2$ increased from a mean baseline value of 37 mmHg to 103 mmHg during NBH and further to 247 mmHg during HBO$_2$.[31] This combined HBO$_2$/NBH therapy–induced increase in $P_{bt}O_2$ corresponded to beneficial outcomes, including an increase in ATP production, decreased hippocampal apoptosis, and increased mitochondrial redox potential.[30, 31] An important finding in the study conducted by Daugherty, et al. was the fact that mitochondrial function was not improved in injured animals after 1 hour of HBO$_2$, but was significantly improved at 4 hours (ie, after the delivery of an additional 3 hours of NBH). This finding suggests HBO$_2$ is acting as a signal transducer that improves mitochondrial function after HBO$_2$ administration and the subsequent administration of NBH enhances this effect.[31]

Functional and cognitive outcomes:

The animals that received the combined HBO$_2$ and NBH treatment performed better in the Morris water maze than did those animals that did not receive treatment.[30]

Safety

Zhou et al. reported no abnormalities in mitochondrial free-radical formation in treated animals.[30]

Summary

The pre-clinical studies evaluating HBO$_2$ that have been conducted over the last 20 years using a variety of animal models have demonstrated benefits in mitochondrial function, neural integrity, lesion volume, and inflammatory response, as well as motor and cognitive outcomes. Thus, they provide clear proof-of-concept evidence supporting the use of HBO$_2$ in the acute treatment of TBI.

Clinical Studies

HBO$_2$ treatment: Phase I

Of the 8 trials that met the inclusion criteria for this review, 2 were Phase I trials. Rockswold et al. recruited 37 patients with a severe TBI and a positive CT scan.[32] These patients underwent an average of five daily 60-minute HBO$_2$ treatments at 1.5 ATA that were initiated within the first 24 hours after injury.[32] Sukoff et al. recruited 50 comatose patients without a surgically correctable lesion, and administered a clinically-dependent

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number of 45-minute HBO$_2$ treatments at 2 ATA.[33] All treatments were instituted within 6 hours of admission and were repeated every 8 hours for 2 to 4 days.

Physiologic outcomes

Both studies found beneficial effects of HBO$_2$ treatment on intracranial pressure (ICP). Rockswold et al. reported that patients presenting with an ICP >15 mmHg had significantly decreased ICPs at both 1 and 6 hours after the HBO$_2$ sessions.[32] Sukoff et al. monitored ICP in 10 patients and found that ICP was reduced in all cases in the chamber.[33] In most cases, lower pressures were sustained for 2 to 4 hours after HBO$_2$.

Cerebral blood flow is normally regulated by cerebral metabolism—so-called metabolic coupling—such that if cerebral oxidative metabolism increases, CBF also increases. Thus, it is of particular note that Rockswold et al. reported that HBO$_2$ improved metabolic coupling; HBO$_2$ significantly increased the cerebral metabolic rate of oxygen (CMRO$_2$) at 6 hours post-HBO$_2$ treatment with a corresponding increase in relatively low pre-treatment CBF.[32]

Functional and cognitive outcomes:

Sukoff et al. reported improvements in awareness and motor activity during treatment in 31 of the 50 patients studied.[33]

Safety

Rockswold et al. reported no permanent sequelae related to HBO$_2$ in any of the patients treated. Sukoff et al. found no pulmonary complications due to suspected toxic effects of HBO$_2$ and no decreased motor function or cognitive awareness compared to patients who received standard care.

HBO$_2$ treatment: Phase II

The remaining 6 studies included in this review were Phase II trials, including patients with Glascow Coma Scale (GCS) scores ranging from 3 to 12. Patients in these studies underwent between 1 and 42 treatments at a range of 1.5 to 2.5 ATA for a duration of 20 to 90 minutes. A majority of these 6 studies initiated treatment within the first few days after the injury.

Physiologic outcomes

Rockswold et al. reported positive metabolic treatment effects of HBO$_2$ compared with the standard of care in terms of improvements in CMRO$_2$, CBF, and P$_{bt}$O$_2$, as well as

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dialysate lactate concentrations and the lactate pyruvate ratio (LPR).[34] This study replicated previous findings demonstrating a reduction in intracranial hypertension in HBO_2-treated patients compared with those that received standard treatment.[32, 35]

Functional and cognitive outcomes

There have been conflicting results regarding the functional outcome of patients who are treated with HBO_2. Lin et al. found that Glasgow Outcome Scale (GOS) scores were improved at 6 months in a subset of patients treated with HBO_2,[36] and Prakesh et al. reported improvements in hospital stay, social behavior, and disability.[37] Holbach et al. reported improved mortality rates at day 10 post-injury and larger rates of complete recovery in HBO_2 treated patients.[38] Artru et al. reported improvements in coma status at 1 month and mortality at 1 year for a subset of severely injured patients.[39] Furthermore, 2 studies found improved GCS scores between study groups.[36, 37]. However, Rockswold, et al. reported no differences in favorable outcome as measured by dichotomized GOS scores at 6 months post-injury between those who received HBO_2 compared to the standard of care.[35] In this prospective, randomized clinical trial 84 patients served as a control group and 84 patients received HBO_2 at 1.5 ATA for 60 minutes. The HBO_2 treatments were given every 8 hours for 14 days unless the patient began to follow commands or became brain dead. In retrospect, the protocol for this clinical outcome study was chosen arbitrarily, and while it was not shown to improve clinical outcome, it did result in a 50% relative reduction in mortality. This reduction in mortality was especially dramatic in patients with negative outcome predictors such as intracranial hypertension, evacuated mass lesions, and GCS scores of 4 to 6.

Safety

Rockswold et al. found no change in CSF F2-isoprostane (a marker of lipid peroxidation) or bronchial alveolar lavage (BAL) IL-6 and IL-8 levels after HBO_2 treatment, indicating no cerebral or pulmonary O_2 toxicity resulting from treatment.[34] In addition, there was no increased incidence of pneumonia, FiO_2 requirements >50%, or positive end expiratory pressure (PEEP) >10 mm H_2O. Artru et al. interrupted individual treatments in 5 cases due to onset of pulmonary symptoms, but these symptoms were transient and may have correlated with improved post-treatment neurological condition.[39]

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Combined HBO$_2$ and NBH treatment

One study has investigated the combined effects of HBO$_2$ and NBH in the clinical setting.[40] Rockswold et al., using the rationale based on the results of the experimental study described above,[31] randomized 42 patients with non-penetrating, severe TBI (GCS, 3-8) and a Marshall classification of ≥2 to either a standard or HBO$_2$ treatment group. Three daily treatments were initiated within 24 hours of the injury and each included 1 hour of HBO$_2$ at 1.5 ATA followed by 3 hours of NBH.

Physiologic outcomes

Brain tissue oxygen tension was elevated during treatment in both relatively uninjured brain tissue was well as pericontusional tissue, and remained elevated after treatment for 2.5 hours, compared with patients that received the standard of care. Intracranial pressure, as well as cerebral dialysate concentrations of glycerol and lactate and dialysate LPR, were decreased in patients who received HBO$_2$ compared with those who received the standard of care. Overall, the reported physiologic outcomes showed positive metabolic effects of treatment in both relatively non-injured and pericontusional areas of brain.

Functional and cognitive outcomes

Both functional outcome and mortality were significantly improved at 6 months post-TBI in the treatment group compared with patients who received the standard of care. The mortality rate at 6 months post-TBI was improved by an absolute 26% (p = 0.04), and a favorable outcome based on the injury severity-adjusted GOS score was improved by 38% (p = 0.02). The results indicate that combining HBO$_2$ and NBH into a single treatment has a potentially synergistic therapeutic effect.

Safety

This study reported reductions in microdialysate glycerol (a marker of phospholipid degeneration in neural tissue cell membranes) and CSF F2-isoprostane levels in those patients who received combined HBO$_2$ and NBH treatment compared with control-treated patients. This finding is important, because it signifies a protective effect against cerebral O$_2$ toxicity related to improved mitochondrial energy production. In addition, there were no reported increases in the incidence of pneumonia, FiO$_2$ requirements >50%, or PEEP >10 cm H$_2$O for the treated group compared with the control group.

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Summary

Overall, the clinical studies reviewed here provide evidence for the potential clinical utility of HBO_2 in the acute stage of severe TBI. These Phase I and II clinical trials demonstrate that increased O_2 availability results in reductions in intracranial hypertension and improvement in oxidative metabolic function, while definitive improvements in functional clinical outcome have been inconsistently demonstrated.

DISCUSSION

Mechanism of HBO_2

During the acute phase of a severe TBI, the metabolic demands of the brain increase but O_2 delivery to the brain decreases due to a reduction in CBF as well as barriers to O_2 diffusion caused by capillary endothelial edema, which is exacerbated by the neuroinflammatory response to trauma, capillary collapse, and increased ICP.[8] This O_2 deficiency forces a conversion to anaerobic metabolism, leading to the depletion of cellular energy (ATP) and eventually to cell death.[41] This phenomenon was observed in the studies reviewed above that report decreased $CMRO_2$, decreased ATP production and increased lactate concentrations in both microdialysate and CSF. The cellular energy crisis resulting from inadequate O_2 delivery results in electrolyte imbalances, stemming from the lack of energy for normal Na^+/K^+ ATP-ase pump function within neurons and glial cells. This imbalance leads to an increased calcium influx, resulting in an abnormally elevated release of excitatory neurotransmitters and further disruption of mitochondrial metabolism in a positive feed-forward manner that causes excessive free-radical build-up. As the neuroinflammatory response continues, apoptosis-mediator proteins such as bcl-2 and bcl-xL initiate the process of cell death.

This biochemical cascade resulting in potentially large-scale cell deathdemonstrates the need for providing an adequate O_2 supply following TBI in order to limit secondary ischemic injury. It is currently unclear whether the benefit seen with HBO_2 is due to a defined threshold of $P_{bt}O_2$ that must be reached (preliminary evidence suggests this threshold may be >200mmHg[34]) or an area under the curve of O_2 dosage that must be reached. Either way, providing an adequate O_2 supply is a task that HBO_2 appears to accomplish. The effects of HBO_2 are mediated by increasing the O_2 dissolved in plasma, as

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opposed to the O_2 carried by hemoglobin. For example, the dissolved O_2 content (volume %) at room air (1 ATA) is 0.32. At 1.5 ATA, it is increased by a factor of 10.[42] When additional O_2 becomes available for diffusion across capillary endothelium, anaerobic metabolism converts back to aerobic metabolism, allowing mitochondria to restore depleted cellular energy.[31] This neuroprotective effect can be objectively observed in the traumatized human brain by improved $CMRO_2$ measurements following HBO_2 treatments, as mitochondrial metabolism accounts for >90% of O_2 consumption in the brain.[32, 34] This neuroprotective increase in $CMRO_2$ leads to a number of physiologic benefits. First, returning to aerobic metabolism results in improved energy production and halts the cascade toward cell death described above. Second, the averted energy crisis allows for a return of normal autoregulation, which can normalize CBF and ICP.[32] Third, it decreases the neuroinflammatory response that leads to apoptosis.[23, 25, 43-46] Fourth, as ATP becomes available from restored mitochondrial function, the function of Na^+/K^+ ATP-ase pumps improves, allowing osmotic effects to alleviate endothelial swelling and edema. In turn, this reverses induced barriers to the diffusion of O_2 to the mitochondria.[47-49] Finally, the BBB stabilizes and there are increases in stem cell production.[24, 47, 48, 50, 51]

Hyperbaric oxygen therapy has also been proposed as a treatment for the chronic sequelae of TBI, but evidence to support HBO_2 for this purpose is weak. Previous review articles have suggested that issues with methodology and statistical analysis may be underlying reasons,[52] but the biochemical mechanism responsible for the benefits of HBO_2 in chronic mild TBI are not well documented in either clinical or pre-clinical work. In our review of the literature, we were able to identify only two pre-clinical studies evaluating HBO_2 for chronic TBI.[15, 51] Notably, the mechanism for any benefit seen with HBO_2 in the treatment of chronic mild TBI is unlikely to be similar to that underlying the acute effect of HBO_2, because the acute mechanism relies on cascades relating to the energy crisis that occurs in the body within hours or days of a severe TBI.

Controversy Surrounding the use of HBO_2

The biochemical mechanisms and physiologic benefits of acutely administered HBO_2 for severe TBI provide objective evidence supporting the use of this treatment in the clinical setting. However, controversy still exists due to safety concerns of an increased O_2

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dose, how meaningful the benefits in functional outcome are, the feasibility of implementing these treatments, and the apparent inability to consistently replicate data.

Safety

One safety concern related to the therapeutic use of HBO_2 in TBI stems from O_2 toxicity, which is caused by oxidative stress and the formation of reactive O_2 species in the lungs and brain tissue after prolonged exposure to O_2.[34, 57] Oxygen toxicity is commonly measured in increments of unit pulmonary toxicity dose (UPTD), which is a theoretical method for calculating relative O_2 doses.[58] One UPTD is equal to 1 minute of exposure to 100% O_2 at 1 ATA, and appropriate conversion factors allow one to quantitate the pressure of O_2 exposure. In general, it is recommended that total O_2 exposure during a single treatment be limited to ≤615 UPTD. The extreme upper limit of a single O_2 exposure is 1425 UPTD, which will produce a predicted 10% decrease in vital capacity in a healthy individual. A treatment consisting of 60 minutes of HBO_2 at 1.5 ATA with compression/decompression at 2 feet/minute generates 130 UPTD. At a pressure of 2.5 ATA, using the same procedure, the O_2 dose is 296 UPTD. Both paradigms are well below the accepted upper limit. It is important to note that interruptions in O_2 exposure between treatments have been shown to increase O_2 tolerance and improve safety; for example, 600 UPTD per day in 2 treatment sessions was administered for weeks without any evidence of accumulative pulmonary toxicity.[59]

Feasibility

Questions have been raised regarding the feasibility of HBO_2, because its use requires hospitals to purchase chambers. However, a higher-cost, multiple-occupancy, large compartment chamber requiring sophisticated operation is not necessary for most hospitals. A lower-cost monoplace chamber, which allows for the treatment of a single patient with external support, is entirely adequate and can be incorporated into a critical care area.[34, 60] Further, it has the advantages of minimal physical space requirements and minimal operation demands, which can be met by training support staff already employed by the hospital, a lack of iatrogenic sickness to the support staff, and a lower cost of purchase and installation. Given the widespread demographic that TBI affects, the wide-scale implementation of an effective treatment option for these severely injured patients should be seen as an investment rather than a cost.

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In addition to the expense, expanding this complex, labor-intensive treatment to multiple centers could be problematic. Experience at Hennepin County Medical Center has demonstrated that HBO_2 can be delivered to patients with severe TBI safely. Over 1900 HBO_2 treatments have been delivered to 167 patients over the course of 4 clinical trials without negative permanent sequalae.[32, 34, 35, 40, 60] As with any new medical procedure, the process has to be taught to other centers, but novel clinical trials can drive practice if new treatments show beneficial effects in randomized trials.

Mixed results

A major concern of implementing HBO_2 as a clinical treatment arises from the perception that the data are not consistently replicated in the literature; 2 main factors may contribute to these inconsistencies. The first factor is the heterogeneous pathophysiology of TBI. Hyperbaric oxygen therapy may not be the best choice for all patients that present with a severe TBI. Studies using subgroup analyses have shown that some patients respond better to treatment than others, such as patients who have lower baseline CBF levels, higher ICP levels, those whose injuries are more severe, and those with mass lesions.[32, 35, 39] Second, suboptimal and inconsistent methodologies have been employed in HBO_2 studies; examples include studies of patients with injuries that vary substantially in severity, and those with poorly defined inclusion criteria, studies that do not consistently randomized patients or blind the analysis, and studies with a high risk for bias.[61] In fact, only 1 study has met the standards of a prospective, randomized, controlled trial.[41] Further complicating this issue, treatment protocols have varied greatly from study to study, resulting in patients receiving variable O_2 dosages initiated at various time-points following injury with sporadic frequencies.

Despite methodological inconsistencies and subsequent incapability to conduct a meta-analysis, this review summarizes data that indicate the positive potential of HBO_2 for the treatment of TBI during the acute post-injury period. However, optimal treatment paradigms are unable to be further delineated at present, because pre-clinical investigators working with TBI models and HBO_2 have used pressures varying from 1.5 to 3.0 ATA, and clinical investigators have used pressures varying from 1.5 to 2.5 ATA. In addition, the lungs of severe TBI patients are frequently compromised by direct lung injury and/or acquired ventilator-associated pneumonia and are therefore susceptible to O_2

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toxicity. Working with those constraints, it is essential to determine the most effective HBO_2 treatment paradigm without producing O_2 toxicity and clinical complications. The ideal HBO_2 treatment paradigm would include pressure (ATA) parameters and information regarding whether NBH delivered after HBO_2 treatment enhances clinical effectiveness. A recently funded randomized clinical trial, the HOBIT trial, will have 2 principal aims: 1) to select the combination of HBO_2 treatment parameters that is most likely to demonstrate improvement in good neurological outcome at 6 months following severe TBI in a subsequent confirmatory trial, and 2) to determine whether there is a >50% probability of the selected HBO_2 treatment demonstrating significant improvement in good neurological outcomes at 6 months following severe TBI in a subsequent confirmatory trial.[9] Based on the previous work described in this review, a targeted subset of patients with severe TBI will be enrolled in the trial.

CONCLUSION

This systematic and comprehensive literature review demonstrates that, despite the controversy surrounding HBO_2 for the treatment of TBI, this therapy has significant clinical potential. Nearly 50 years of pre-clinical and clinical research demonstrate a possible beneficial effect of this treatment, yet acutely administered O_2 therapy is still considered an experimental procedure. Because of this, the HOBIT trial, a recently NINDS-funded, adaptive Phase II clinical trial is warranted, and it is anticipated that an optimal treatment paradigm for potential efficacy will be established from these data.

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Table 1. Point-Based Ranking System

A. Clinical Studies

Type	Description	Point Value
Control Adequacy	(1) Matched on Gender (2) Matched on Age (3) Matched on another variable	0-3
Power Analysis	Is one present?	0-1
Description of Statistics	Is a description present?	0-1
Blinding	(1) Single Blind (2) Double Blind	0-2
Randomization	Where subjects randomized?	0-1
Dose-Response Results	Are they present?	0-1
Total Possible		**9**

B. Pre-Clinical Studies

Type	Description	Point Value
Control Adequacy	(1) Matched on Gender (2) Matched on Age or Size (3) Matched on another variable	0-3
Power Analysis	Is one present?	0-1
Description of Statistics	Is a description present?	0-1
Blinding	Blinded Assessment	0-1
Randomization	Where subjects randomized?	0-1
Dose-Response Results	Are they present?	0-1
Total Possible		**8**

This table demonstrates how point values were assigned for the purpose of objectively

ranking the methodology of (A) clinical studies and (B) pre-clinical studies.

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Table 2: Pre-Clinical Data on HBO used for Acute TBI

Author & Year	NINDS Criteria Ranking	Animal Type/ Eligibility (genetic)	Injury and Treatment Groups; N	Adequacy of Controls	Blinding and Randomizing Methodology	FiO2 at ATA; Window post-TBI; Duration; Frequency	Positive Treatment Effects	Negative Treatment Effects	Neutral Treatment Effects
Wee et al., 2015	5	Sprague-Dawley rats (male, 290-310 g)	(1) LFP + HBO; ≥6 (2) LFP; ≥6 (3) Sham Surgery; ≥6	Gender- and size-matched non-treatment and sham surgery groups	Blinded assessment of brain tissue; Randomized into groups	100% at 2 ATA; "immediate"; 60 min; One Treatment Sacrifice at 72 hr	• TNF-α (Reduced number of positive stained cells in Group 1 compared to Group 2*; Attenuated serum concentration in Group 1 compared to Group 2*) • Perilesional Neuronal Damage (Reduced in Group 1 compared to Group 2)	•	•

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| | | | | | | | • Perilesional Apoptosis (Reduced in Group 1 compared to Group 2*)

• TGIF Expression in Neurons (Reduced number of expressing neurons in Group 1 compared to Group 2*)

• TGF-B1 Expression in Neurons (Reduced number of expressing neurons in Group 1 compared to Group 2*)

• Motor Function (Elevated run speed in Group 1 compared to | | |

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Study		Animal	Groups	Controls	Blinding	HBO Protocol	Outcomes		
							Group 2*)		
Chen et al., 2014	4	C57BL/6 WT mice (female, 8 weeks old)	(1) CI + HBO; 9 (2) CI; 9 (3) Sham + HBO; 9 (4) Sham Surgery; 9	Gender- and age-matched non-treatment groups	Blinded functional assessments; NM	100% at 2 ATA; 3 hr; 1 hr; Daily treatments for 5 days	• Serum and Ipsilateral Cortex IL-10 Concentration (Elevated in Group 1, 2, and 3 compared to Group 4*; Elevated in Groups 1 and 3 compared to Group 2*; Groups 1 and 3 remain elevated at 6 hours post-HBO*, but not at 12 hours)	•	•
Kraitsy et al., 2014	4	Sprague-Dawley rats (male, 320-330 g)	(1) Severe[1] LFP + HBO; 12 (2) Severe[1] LFP; 9 (3)	Gender- and size-matched sham and non-treatment	Blinded Analysis; NM	100% at 2.2 ATA; 1 hr; 1 hr; Daily treatments on post-	• Cortical Lesion (MRI-measured HBO-associated reduction*) • SSEP (HBO-associated reductions in CCT by day 22 in the	•	•

| | | | Moderate[1] LFP + HBO; 12 (4) Moderate[1] LFP; 9 (5) Sham-operated; 7 | groups | | injury days 1-3, 7-9, 13-15, and 19-21 | ipsilateral hemisphere*) • MBP Isoform Expression (Elevated in treated animals compared to untreated at Day 16 and 22*; MBP Isoform expression in treated animals associated with remyelination in ipsilateral cortex*) • Behavioral Tests (HBO-associated improvements in function by day 22 in three of four tests*) | | |
| Wei et al., 2014 | 4 | New Zealand | (1) CI + HBO; 15 | Size- and Age- | Blinded Clinical | 100% at 2.5 ATA; ~4 | • VCS (Group 1 scores were higher at 30 days | • | • Contra lateral ADC |

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| | | White Rabbits (3-4 months, 2.5-3 kg) | (2) CI; 12 (3) Sham + HBO; 6 (4) Sham Surgery; 6 | matched sham and non-treatment groups | outcome assessment; Randomized into groups | hours; 60 min; Twice a day for first 3 days and once a day for 4 subsequent days | post-injury than at 1 day post-injury; Group 1 scores were higher than Group 2 at 30 days post-injury*)
• Focal BBB Dysfunction (Reduced dysfunction of Group 1 compared to Group 2 at 1, 3, 7, and 30 days post-injury*; Dysfunction peaked at 3 hr post-injury and was normal by Day 7 for Group 1*)
• Perifocal BBB Dysfunction (Reduced dysfunction in Group 1 compared to Group 2 and 3 and 7 days post- | | and BBB Dysfunction (No difference between Group 1 and Groups 3 or 4) |

							injury*; Dysfunction peaked at 1 day and was normal by Day 3 for Group 1*) • Focal ADC Changes (Elevated values for Group 1 compared to Group 3 at 3 hours, 1 day, and 30 days post-injury*, but Reduced at 3 and 7 days post-injury*; Elevated values for Group 1 compared to Group 2 at 1, 3, and 7 days post-injury*, but Reduced at 30 days post-injury compared to Group 2*)		

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							• Perifocal ADC Changes (Elevated values for Group 1 compared to Group 3 at 1, 7, and 30 days post-injury*; Reduced values compared to Group 2 at 1, 3, and 30 days post-injury*)		
Yang et al., 2014	4	Sprague-Dawley rats (male, 300-350 g)	(1) LFP + HBO-early-delayed; 90 (2) LFP + HBO-early; 90 (3) LFP; 90 (4) Sham Surgery; 90	Gender- and size-matched sham and non-treatment groups	NM; NM	(1) 1.5 ATA; 6 hr; 1.5 hr; 15 daily treatments for days 1-15 and 60-74 (2) 1.5 ATA; 6 hr;	• Hippocampal Apoptosis (Decreased TUNEL-positive cells associated with timing of HBO; least amount of apoptosis in Group 1*) • Morris Water Maze (Decreased time associated with timing	•	•

| | | | | | | 1.5 hr; daily for 15 days | of HBO; quickest rats were those in Group 1*)
• Brain Water Content (decreased in Groups 2 and 1 at day 15*)
• BBB Dysfunction (Reduced permeability in Groups 1 and 2 at day 15*)
• Hippocampal HIF-1α (Decreased after HBO treatment in all groups*) | | |
| Zhang et al., 2014 | 5 | New Zealand White Rabbits (male, 2- | (1) bTBI + HBO (2) bTBI (3) Sham blast | Gender- and size-matched non-treatment | Blinded data collection and analysis; Randomized into | 100% at 2 ATA; 12 hr; 60 min; One Treatment | • NAA/Cr (Groups 1 and 2 had reduced values at 12 and 24 hours compared to Group 3*; Group 1 was no | • | • |

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| | | 2.5 kg) | Total N = 146 | and sham injury groups | treatment groups | | different than Group 3 at 7 days, but Group 2 maintained reduced values*)

• Cho/Cr (Group 1 elevated at 6 hours*, reduced at 12 hours, and elevated at 24 hours* compared to Groups 2 and 3; Neither Group 1 or 2 was different from Group 3 at 7 and 14 days)

• Water Content of Brain Tissue (Increases after injury in Groups 1 and 2 over Group 3*; Group 1 values | | |

							were consistently reduced compared to Group 2 at all time points*) • BBB Dysfunction (Reduced permeability in Group 1 compared with Group 2 in ipsilateral cortex at 24 and 48 hours post-injury*) • Inflammatory mRNA Expression (In Group 1 compared to Group 2: Caspase-3 reduced at 3 days post-injury*; IL-8 reduced at 12 hours post-injury*; TNF-a reduced at 12 and 24		

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							hours post-injury*) • Inflammatory Protein Expression (In Group 1 compared to Group 2: Caspase-3 reduced at 3 days post-injury*; IL-8 reduced at 12 and 24 hours post-injury*; TNF-α reduced at 12 hours post-injury*)		
Lim et al., 2013	4	Sprague-Dawley rats (male, ~300 g)	(1) LFP + HBO at 1 hour post-injury; 6 (2) LFP + HBO at 8 hr post-injury; 6 (3) LFP; 6	Gender- and size-matched non-treatment and sham surgery groups	NM; Randomized into groups	100% at 2 ATA; 1 or 8 hr for Group 1 and 2, respectively; 60 min; 1 treatment	• Motor Dysfunction (Groups 1 and 2 showed similar increased functionality compared to Group 3*) • Infarction Volume (Groups 1 and 2 showed similar	•	• No difference between Groups 1 and 2 in any variable tested.

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| | | | (4) Sham Surgery; 6 | | | Sacrifice at 72 hr post-injury | decreases in volume compared to Group 3*)
 • Perilesional Apoptosis (Similar decreases in TUNEL-positive cells in Groups 1 and 2 compared to Group 3*)
 • Microglial Activation (Groups 1 and 2 showed similar reductions in perilesional cortex microglial activation at 72 hr post-injury compared to Group 3*)
 • TNF-α Expression | | |

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							(Similar reduced expression in perilesional microglial of Groups 1 and 2 compared to Group 3*)		
Liu et al., 2013	4	Sprague-Dawley rats (male, 300-350 g)	(1) CI + HBO; 10 (2) CI; 10 (3) Sham Surgery; 10	Gender- and size-matched non-treatment and sham surgery groups	NM; Randomized into groups	100% at ~2 ATA; 6 hr; 1 hr; Daily treatments for 2 weeks	• Morris Water Maze (Group 1 performed better than Group 2 at 1 and 2 weeks post-injury*) • Hippocampal NAA/Cr (Group 2 had reduced ipsilateral levels compared to Groups 3* and 1 at all time points, while Group 1 values were elevated over Group 2 at 48	•	• Hippocampal Metabolism (no difference between groups in NAA/Cho and NAA/Cr ratios in contralateral CA3)

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| | | | | | | | hours, 1 week and 2 weeks post-injury*)

• Hippocampal NAA/Cho (Groups 1 and 2 had reduced values at all time points compared to Group 3*, but Group 1 levels were elevated compared to Group 2 at 2 weeks post-injury*)

• Hippocampal Histology (Group 1 had tighter distributed neurons and increased Nissl bodies in the CA3 region compared to Group 2 at 2 weeks | | |

							post-injury) • Hippocampal GFAP Expression (Decreased in Group 1 compared to Group 2 at 2 weeks post-injury*)		
Brkic et al., 2012	5	Wistar rats (male, ~250 g)	(1) SMSA + HBO; 8 (2) SMSA; 8 (3) Sham Surgery + HBO; 8 (4) Sham Surgery; 8 (5) HBO; 8 (6) Control; 8	Gender- and size matched non-treatment, sham surgery, and no surgery groups	Blinded Evaluation; Randomized to groups	100% at 2.5 ATA; 5 hrs; 1 hr; Daily treatments for 10 days	• Locomotor Coordination (Groups 1 and 2 showed worse coordination at days 3 and 7 than Groups 3-6*; Group 1 showed better coordination than Group 2 on day 10*; No difference between Group 1 and Groups 3-6 on day 10) • Grip Strength (Reduced on days 3, 7,	•	•

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| | | | | | | | and 10 on contralateral side in Group 1 and 2 compared to Groups 3-6*, but Group 1 was stronger than Group 2 on Day 10*)
• Sprouting (Elevated after 10 days of HBO treatment)
• Synaptogenesis (Large amount of synaptogenesis after 10 days of HBO) | | |
| Lin et al., 2012 | 3 | Sprague-Dawley rats (male, 276-333 | (1) LFP + HBO; ≥8
(2) LFP; ≥8
(3) Sham surgery; ≥8 | Gender- and size-matched non-treatment | NM; NM | 100% at 2 ATA; 3 hr; 1 hr; Two times per day for 3 | • Behavioral Test (Groups 1 and 3 performed better than Group 2*)
• Cognitive Test (Groups | • | • |

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| | | g) | | and sham surgery groups | | days

Behavioral testing and sacrifice done at 4 days post-injury | 1 and 3 performed better than Group 2*)

• Infarction Volume (Reduction in volume of Groups 1 and 3 compared to Group 2*)

• Neuron and Glial Apoptosis (Less cell death in Group 1 compared to Group 2*)

• Gliosis (Reduced number of perilesional astrocytes in Groups 1 and 3 compared to Group 2*)

• Neuronal Loss (Less active neurons in | | |

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| | | | | | | | Group 2 compared to Groups 1 and 3*)
• Neurogenesis (Elevated neurogenesis in Group 1 compared to Group 2*)
• Angiogenesis (Elevated number of newly forming endothelial cells in Group 1 compared to Group 2*)
• Inflammation (Lower levels of inflammation measured by serum TNF-α, MPO activity, and IL-10 in Group 1 than Group 2*) | | |

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| Wang et al., 2010 | 6 | Sprague-Dawley rats (male, 250-280 g) | (1) CI + HBO; 6 (2) CI + HBN; 6 (3) CI; 6 | Gender- and size-matched non-treatment groups | Blinded Neurologic evaluations; Randomized into groups | (1) 100% at 3 ATA (3) 7% at 3 ATA; (1 and 3) 6 hr; 1 hr; One Treatment-Sacrifice at 48 hr | • Lesion Severity (Group 1 had less severe lesions than Groups 2 and 3 at 48 hours post-injury) • Hippocampus Neurons (Elevated number of neurons in the CA2 region of Group 1 compared to Groups 2 and 3*) | • | • |
| | | | (1) CI + HBO at 6 time points post-injury; 96 (2) CI; 12 (3) Sham Surgery; 12 | Gender- and size-matched non-treatment and sham surgery groups | | 100% at 3 ATA; a. 3 hr, b. 6 hr, c. 12 hr, d. 24 hr, e. 48 hr, f. 72 hr; 1 hr; One treatment- | • Neurologic Function (Elevated Function in Groups 1a, 1b, and 1c compared to Groups 1d, 1e, 1f, and 2*) • Brain Water Content (Reduced water content in Groups 1a, | • | • |

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| | | | | | | Sacrifice at 4 days | 1b, and 1c compared to Groups 1d, 1e, 1f, and 2*)
• Hippocampal Neurons (Elevated number of neurons in CA2 region of Groups 1a and 1b compared to Groups 1c, 1d, 1e, 1f, and 2*; Elevated number of neurons in CA2 region of Group 1c compared to Groups 1d, 1e, 1f, and 2*) | | |
| | | | (1) CI + HBO for 1 treatment; 40
(2) CI + | Gender- and size-matched non-treatment | | 100% at 3 ATA; a. 3 hr, b. 6 hr, c. 12 hr, d. 24 hr, e. 48 | • Neurologic Function (Similar elevated Function in Groups 1b, 2b and 3b compared to Group 4*; Similar | • | • Neurologic Function (Groups 1f, 2f, and 3f |

| | | | HBO for 3 treatments ; 40 (3) CI + HBO for 5 treatments ; 40 (4) CI; 40 | groups | | hr, f. 72 hr; 1 hr; Either 1, 3 or 5 Treatments - Sacrifice at 9 days | elevated function in Groups 2d/e and 3d/e compared to Groups 1d/e and 4*; Greater elevation in function of Groups 1a and 1b than Groups 2e and 3e*) • Hippocampal Neurons (Similar elevated number of neurons in CA2 region of Groups 1b, 2b and 3b compared to Group 4b*; Similar elevated number of neurons in CA2 region of Groups 2d/e and 3d/e compared to Groups | | did not show improveme nt compared to Group 4) |

							1d/e and 4*; Greater elevation in number of CA2 neurons of Groups 1a and 1b than Groups 2e and 3e*;)		
			(1) CI + HBO; 8 (2) CI; 8 (3) Sham Surgery; 8	Gender- and size- matched non- treatment and sham surgery groups		100% at 3 ATA; 6 hr; 1 hr; One Treatment- Sacrifice at 24 hr	• Apoptosis (TUNEL-positive cells were elevated in Group 2 compared to Group 3*, but reduced in Group 1 compared to Group 2*) • Bcl-2 (Decreases seen in Group 2 compared to Group 3* were recovered in Group 1*) • Caspase-3 (Increases seen in Group 2	•	• Bax (No difference between groups)

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							compared to Group 3* were reduced in Group 1*)		
Palzur et al., 2008	4	Sprague-Dawley rats (male, 300-350 g)	(1) DCD + HBO; NM (2) DCD; NM (3) Sham Surgery; NM	Gender- and size-matched sham and non-treatment groups	Blinded Analysis; NM	100% at 2.8 ATA; 3 hr; Two sessions of 45 min with 5 min break; Repeated at 24 hr after injury	• Neuronal Density (Enhanced neuronal survival and axonal architecture in Group 1 compared to Group 2*) • Transmembrane Mitochondrial Potential (Reduced loss of potential in Group 1 compared to Group 2*) • Caspase-3 Activity (Reduced in Group 1 compared to Group 2*)	•	• Caspase-8 Activity (no difference seen between Groups 1 and 2)

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							• Caspase-9 Activity (Reduced in Group 1 compared to Group 2*)		
Soustiel et al., 2008	3	Sprague-Dawley rats (male; 300-350 g)	(1) DCD + HBO; 8 (2)DCD; 8 (3) Sham-Surgery; 8	Gender- and size-matched sham and non-treatment groups	NM; Fields of injury were randomly chosen for histological analysis	100% at 2.8 ATA; 3 hr; 2 consecutive sessions of 45 min (5 min break); 2nd treatment at 24 hours post-injury	• TSPO Expressing Cells (Reduced expression in Group 1 compared to Group 2*) • Mitochondrial Transmembrane Potential (Restored 70% of lost potential caused by injury; Injection of PK11195 prevented restoration of potential) • Caspase-9 Activity (Reduced in Group 1 compared to Group	•	• GFAP Expressing Cells (No difference in percentage of cells between Groups 1 and 2)

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| Voigt et al., 2008 | 3 | Sprague-Dawley rats (male, 250-300 g) | (1) CI + HBO; 5 (2) CI; 5 | Gender- and size-matched non-treatment group | NM; NM | 100% at 2.5 ATA; 1 hr; 1 hr; 1 treatment | 2*; Reduction prevented by injection of PK11195*; Associated with loss of transmembrane mitochondrial potential)

 • MRI-Observed Lesion Volume (Reduced in Group 1 compared to Group 2 at 24 hr post-injury*; Group 1 continued to decrease from 24 to 72 hours*)
 • Relative ADC in ipsilateral vs. contralateral side of the injury (Elevated in Group 2 compared to | • | • |

							Group 1 at both 24 at 72 hours*; No change seen in either group between 24 and 72 hours)		
Liu et al., 2006	4	Sprague-Dawley rats (male, 250-300 g)	(1) CI + HBO; 20 (2) CI; 20	Age-, Size-, and gender matched non-treatment group	NM; Randomized to treatment	95% at 2.5 ATA; < 30 min; 30 min; one treatment before sacrifice at either 3, 6, 12, 24, or 72 hr post-injury	• Cyt C (Reduced at 3, 6, 12, and 24 hours in Group 1 compared to Group 2*) • Bcl-2 (Elevated at all time points in Group 1 compared to Group 2*) • Bax (Reduced at 3, 6, 12, and 24 hours in Group 1 compared to Group 2*) • Mitochondria (Swollen and vague matrixes at	•	•

							3, 6, 12, and 24 hours after TBI in Group 2 compared to Group 1)		
Vlodavsky et al., 2006	3	Sprague-Dawley rats (370-430 g)	(1) DCD + HBO; 20 (3) DCD; 10	Size-matched; Non-treatment group after injury	Blindly Assessed Pathology; NM	100% at 2.8 ATA; 3 hr; two 45 min sessions with 5 min break; daily treatments for three treatments	• Perilesional Apoptosis (Group 1 post-treatment TUNEL staining showed reduced apoptosis in injured tissue compared to Group 2*) • Inflammatory Response (Reduced post-treatment neutrophil infiltration compared to Group 2*) • MMP-9 Expression (Reduced post-	•	• MMP-2 and TIMP-1 Expression (no difference seen between groups)

							treatment compared to Group 2*)		
Vlodavsky et al., 2005	3	Sprague-Dawley rats (370-430 g)	(1) DCD + HBO (2) DCD + Hypoxemia + HBO (3) DCD + Hypoxemia (4) DCD Total N= 50	Size-matched non-treatment groups	NM; NM	100% at 2.8 ATA; 3 hr; Two 45 min sessions with a 5 min break; Twice a day for 3 days Hypoxemia: 60 min at 1 ATA with variable FiO$_2$ to	• Bcl-2 and Bcl-xL (Reduced pre-treatment levels in Groups 2 and 3 compared to Groups 1 and 4; Increased levels after treatment in Groups 1 and 2*) • Bax (Elevated after post-traumatic hypoxemia; Decreased staining intensity after treatment) • Perilesional Apoptosis (Decreased TUNEL-positive cells in Groups 1 and 2	•	•

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						maintain SaO_2 at 82-86%	compared to Groups 4 and 3, respectively*)		
Palzur et al., 2004	4	Sprague-Dawley rats (male; 370-430 g)	(1) DCD + HBO; 5 (2) DCD + hypoxia + HBO; 5 (3) DCD + hypoxia; 5 (4) DCD; 5	Gender- and size-matched non-treatment group	NM; NM	100% at 2.8 ATA; 3 hr; 2 consecutive sessions of 45 min (5 min break); daily for 3 days Hypoxemia: SaO_2 = 82-86%; NM; 60 min; 1 treatment	• Perilesional Apoptosis (Reduced in Group 1 compared to Group 4*; Reduced in Group 2 compared to Group 3*; Elevated in Group 3 compared to Group 4*)	•	•

| Tinianow et al., 2000 | 2 | Wistar Rats (male, juvenile) | (1) CI + HBO; 10 (2) CI; 9 | Age- and Gender-matched non-treatment group | NM; NM | 100% at 2.5 ATA for 60 min for first 5 treatments, 100% at 2 ATA for 41 min for 6th treatment, and 100% at 2.5 ATA for 30 min for treatments 7-40; <4 hr window; 4 treatments per day for 10 days | • Beam Walk Test (Group 1 performed better than Group 2 at 3 days post-injury*) • Contusion Surface Area (Reduced area in Group 1 compared to Group 2 at day 10 post-injury*) | • Four animals in Group 1 died after first 5 treatments due to oxygen toxicity • Some animals lost function of ipsilateral forepaw | • Morris Water Maze (No difference between Groups in post-injury testing) • Beam Walk Test (No difference between Groups at 6 and 9 days post-injury) • CA3 Region Pyramidal |

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							due to oxygen toxicity.		Cells (No difference between Groups in cell count ratio of contralateral to ipsilateral)
Nida et al., 1995	5	Sprague-Dawley rats (male, 275-400 g)	(1) CI + HBO; 14 (2) CI + hypoxia; 14 (3) CI; 15 (4) FP + HBO; 15 (5) FP + hypoxia;	Size- and gender-matched non-treatment and sham groups	N/A; Randomized to groups	97-99% at 1.5 ATA; 4 hr; 1 hr; 1 treatment Hypoxia: 30 min of 13% FiO_2	•	•	• Cerebral Edema (No difference in amount of edema at the trauma site between Groups 1-3

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			11 (6) FP; 13 (7) Sham; 12						and Groups 4-6)

*Significant Finding; [1]Injury severity defined by pressure of injury impact

ADC= Apparent Diffusion Coefficient; ATA= x1 Atmospheric Pressure; AVO$_2$= Arteriovenous Oxygen Difference; Bax= bcl-2-Associated X Protein; BBB= Blood-Brain Barrier; bcl-2= B-Cell Lymphoma 2; bcl-xl= B-Cell Lymphoma-extra large; bTBI= blast-induced TBI; CCT= Central Conduction Time; Cho= Choline; CI= Cortical Impact; CPP= Cerebral Perfusion Pressure; Cr= Creatine; CSF= Cerebral Spinal Fluid; Cyt C= Cytochrome c; DCD= Dynamic Cortical Deformation; EEG= Electroencephalogram; FiO$_2$= inspired oxygen percentage; FP= Fluid Percussion; g= grams; GFAP= Glial Fibrillary Acidic Protein; HBO= Hyperbaric Oxygen Treatment; HBN= Hyperbaric Nomoxia; HIF-1α= alpha subunit of hypoxia inducible factor; hr= Hours; ICP= Intracranial Pressure; IL= Interleukin; kg= kilogram; KO= Knockout; LFP: Lateral Fluid Percussion; LPR= Lactate Pyruvate Ratio; MAP= Mean Arterial Pressure; MBP: myelin-basic protein; MCP-1= Monocyte Chemotactic Protein-1; min= Minutes; MIP-2= Macrophage Inflammatory Protein-2; MMP-2: Matrix Metalloproteinase-2; MMP-9: Matrix Metalloproteinase-9; MPO= Myeloperoxidase; MRI= Magnetic Resonance Imaging; mRNA= micro ribonucleic acid; N= sample size; N/A= Not Applicable; NAA= N-Acetyl Aspartate; NM= Not Mentioned; PaCO$_2$= Arterial Pressure of carbon dioxide; PaO$_2$= Arterial Oxygen Pressure; PbO$_2$= Brian Tissue Oxygen Pressure; PK11195= TSPO Ligand; PvO$_2$= Venous Oxygen Pressure; SaO2= Arterial Oxygen Saturation; SMSA= Sensorimotor Cortex Suction Ablation; SSEP: somatosensoty-evoked potentials; TBI= Traumatic Brain Injury; TGIF= Transforming Growth Interacting Factor; TGF-B1= Transforming Growth Factor-B1; TIMP-1= Tissue Metallopeptidase Inhibitor 1; TNF-α= Tumor Necrosis Factor-alpha; TSPO= Translocator Protein; TUNEL= Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling; VCS= Veterinary Coma Score; WT= Wild Type; ZO-1= Zonula Occludens-1

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Table 3: Pre-Clinical Data on HBO followed by NBH for Acute TBI

Phase 2 Data:

Author & Year	NINDS Criteria Ranking	Animal Type/ Eligibility (genetic)	Injury and Treatment Groups; N	Adequacy of Controls	Blinding and Randomizing Methodology	Treatment Protocol; Frequency	Positive Treatment Effects	Negative Treatment Effects	Neutral Treatment Effects
Zhou et al., 2007	3	Sprague-Dawley rats (male; 290-350 g)	(1) LFP and HBO + NBH, 23 (2) LFP; 23 (3) Sham Surgery; 22	Gender- and size-matched Sham and non-treatment groups	NM; Random selection of brains for histological analysis	15 min post-TBI window, followed by 100% FiO_2 at 1.5 ATA for 1 hr, followed by 3 hours of 100% FiO_2 at <1 ATA; 1 treatment	• ATP Production (Elevated for Group 1 compared to Group 2*) • Morris Water Maze (Group 1 took less time than Group 2*) • Hippocampal Apoptosis (Group 1 showed less apoptosis in CA2/3 than Group 2*) • No Free Radical	•	•

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							Formation Abnormalities Seen in the Mitochondria		
Daugher ty et al., 2004	3	Sprague -Dawley rats (male, 275-350 g)	(1) LFP + NBH + HBO; 6 (2) Sham + NBH + HBO; 8	Gender - and size- matche d sham injury group	NM; NM	No post-injury window, followed by 30% FiO$_2$ at 1 ATA for 30 min, followed by 100% FiO$_2$ at 1 ATA for 30 min, followed by 100% FiO$_2$ at 1.5 ATA for 30 min; 1 treatment before sacrifice	• PbO$_2$ (HBO treatment elevated levels above that seen at 30% or 100% FiO$_2$ for both injured and sham-injured animals*)	•	• PbO$_2$ (Both injured and sham-injured responded in similar ways to HBO)
			(1) LFP + HBO + NBH; 4 (2) LFP; 4 (3)	Gender - and size- matche d sham and			15 min post-TBI window, followed by 100% at 1.5 ATA for 1 hr, followed by 100% at 1 ATA for 3 hrs; 1	• VO$_2$ (Elevated levels in Groups 1 and 3 compared to Groups 2 and 4, respectively*) • Mitochondrial Redox Potential (Reduced pre-	•	•

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			Sham + HBO + NBH; 4 (4) Sham; 5	non-treatment groups		treatment before sacrifice	treatment levels in Groups 1 and 2 compared to Group 4*; Group 1 pre-treatment values reduced compared to post-treatment values*; Post-treatment Group 1 levels elevated over Group 2* and no different than Group 4 levels)		

*Significant Finding

ATA= x1 Atmospheric Pressure; ATP= Adenosine Triphosphate; FiO$_2$= inspired oxygen percentage; g= grams; HBO= Hyperbaric Oxygen

Treatment; LFP: Lateral Fluid Percussion; min= Minutes; N= sample size; NBH= Normobaric Hyperoxia; NM= Not Mentioned; PbO$_2$= Brian

Tissue Oxygenation; TBI= Traumatic Brain Injury; VO$_2$= Oxygen Consumption

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Table 4: Clinical Data on HBO for Acute TBI

4A: Phase 1 Data:

Author & Year	NINDS Criteria Ranking	Treatment Groups; N	Eligibility	Blinding and Randomizing Methodology	Adequacy of Controls	FiO2 at ATA; Window post-TBI; Duration; Frequency	Positive Treatment Effects	Negative Treatment Effects	Neutral Treatment Effects
Rockswold et al., 2001	1	HBO; 37	Non-penetrating TBI; PR GCS 3-8 or deterioration to ≤ 8 within 48 hours of injury; Marshall Classification > 2; Age 8-84	NM; N/A	Baseline measurements were used as comparisons	100% at 1.5 ATA; 9-49 hr (Average: 23 hours); 1 hr; 2nd treatment was 8 hr after 1st treatment and 5	•CBF (Elevated after treatment in those that began treatment with a low CBF*; Elevated after treatment in those that	•	• No changes seen between pre- and post-treatment levels of $AVDO_2$, Hemoglobin, CPP, and pH

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						additional treatments were provided at 24 hr intervals	began with a normal CBF*; Reduced after treatment in those that began with a high CBF*) •CMRO$_2$ (Elevated after treatment for those that began treatment with low and normal levels of CBF*) •CSF Lactate (Reduced		

							after treatment, N=15*) •ICP (Reduced to below baseline levels in those with high pre-treatment ICP levels*)		
Sukoff et al., 1982	0	(1) HBO with clinically-indicated ICP monitor; 10 (2) HBO without	Coma resulting from TBI; Pupillary abnormalities; No operative intracranial lesions	NM; N/A	Baseline measurements were used as comparisons	100% at 2 ATA; <6 hr after ADM; 45 min; every 4-8 hr for 2-4 days depending on clinical	•ICP (decreased during treatment*) (1) •9/10 patients demonstrated improved awareness		

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| | | ICP monitor; 40 | | | | | response | and motor activity in the chamber (2) •22/40 patients demonstrated improved awareness and motor activity in the chamber | | |

*Significant Finding

4B: Phase 2 Data:

Author & Year	NINDS Criteria Ranking	Treatment Groups; N	Eligibility	Blinding and Randomizing Methodol	Adequacy of Controls	FiO2 at ATA; Window post-TBI; Duration;	Positive Treatment Effects	Negative Treatment Effects	Neutral Treatment Effects

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				ogy		Frequency			
Prakash et al., 2012	2	(1) HBO; 28 (2) Controls; 28	TBI; GCS < 8; No other injuries; Children	NM; Randomly selected control group	Matched control group	100% at NM; 10-12 days; NM; weekly for 3 weeks	• GCS (Elevated after HBO treatment) • Hospital Stay (Shorter stay in HBO group); • Social Behavior (more improvement in HBO group); • Disability (HBO group returned to school earlier)		
Rockswold et al., 2010	6	(1) HBO; 26 (2) Standard of Care; 22	Non-penetrating TBI; GCS 3-8; 1+ Reactive Pupil;	NM; Randomized into treatment groups prospectively	Matched for age, gender, initial GCS score, and CT findings	100% at 1.5 ATA; Average= 19 hr; 1 hr; one treatment every 24 hr for 3	• CMRO$_2$ (Increased from pre- to 1 hour post-treatment in Group 1 for those patients with reduced or normal baseline CBF and		• Dialysate Glucose levels did not change • CMRO$_2$ changes between pre- and post-

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						treatments	continued to increase at 6 hours post-treatment*; Elevated Group 1 post-treatment values compared to Group 2*) • CBF (Elevated in Group 1 after treatment compared to pre-treatment levels and Group 2 levels*) • Dialysate Lactate (Decreased after treatment in Group 1 compared to pre-treatment and to		treatment did not differ in Group 1 compared to Group 2
			Marshall Classification >1; No Prior Severe Brain Injury; Mean age 35 years						

							Group 2*)	
							• PbO_2 (Elevated after treatment in Group 1 compared to pre-treatment levels and Group 2*)	
							• Dialysate LPR (Decreased after treatment in Group 1 compared to pre-treatment levels and Group 2*)	
							• ICP (Reduced post-treatment values in Group 1 compared to Group 2*)	
							• $AVDO_2$ (Reduced post-treatment values compared to	

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							pre-treatment*)		
							• CSF F2-isoprostane levels did not change		
							• BAL IL-6 and IL-8 levels did not change		
Lin et al., 2008	5	(1) HBO; 22 (2) Control; 22	TBI; GCS 3-12; No mult-trauma; spontaneous respiration; Age >15	NM; Prospectively Randomized	Groups matched for age, gender, GCS, diagnosis, and surgical intervention	100% at 2.0 ATA; 27 days; 1.5 hr; 20 days over the course of 4 weeks	• GCS (Elevated compared to controls after HBO treatment*) • GOS (Improvement was better at 6 months for a subgroup of Group 1 compared to Group 2*)		• GOS at 3 months was not different between groups
Rocksw old et	7	(1) HBO; 84	TBI; GCS 3-9	Blinded Outcome	Groups matched	100% at 1.5 ATA; 26 hr;	• ICP (Lower in the subgroup of		• ICP was not different

al., 1992		(2) Standard of Care; 84		Assessment; Randomized to treatment groups	for age, gender, body weight, GCS, and pupil reactivity	1 hr; every 8 hr for 2 weeks or until brain death or until they could obey commands	patients in Group 1 that received a myringotomy compared to those in Group 1 that didn't and Group 2*); • Mortality (Reduced in Group 1 compared to Group 2 at 12 months*); • Mortality (Reduced in Group 1 patients with GCS 4-6 or ICP<20mmHg compared to same subgroups of Group 2*)		between Groups 1 and 2 during HBO treatment • GOS at 12 months was not different between groups
Artru et	3	(1) HBO;	Head	Initial	Age, coma	100% at 2.5	• Improvement in		• Mortality at 1

al., 1976		31 (2) Standard of Care; 29	Injuries in a Coma; Age 5-70	coma severity score assignment was blinded; Randomized to treatment groups	severity, diagnosed brain lesions, and rate of surgical intervention was not different between groups	ATA; 4.5 days; 1 hr; daily for 10 days followed by 4 days of no treatment and another 10 daily sessions	consciousness at 1 month*, reduced rate of persistent coma at 1 month,* and reduced mortality at 1 month and 1 year for those patients <30 years old, not reacting in an adapted manner to painful stimuli, and not operated on • Decreased duration of coma in survivors of Group 1 compared to Group 2		year was not different between groups
Holbach	1	(1) HBO;	Traumati	NM; Every	Matching	100% at 1.5	• Mortality (Group 2		

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		49 (2) Standard of Care; 50	c mid-brain syndrome; Age Range 3-65 (Mean: 22.6 years old)	other admission underwent HBO treatment	was not mentioned	ATA; 2-10 days; 20-30 min; Between 1 and 7 times per patient	had quicker reductions in survival time between day 2 and 7 compared to Group 1; 87% survival rate in Group 1 at day 10 compared to 54% survival rate in Group 2; Largest differences in survival rates between groups is seen in those patients < 30 years old) • Recovery (Complete recovery seen in		
et al., 1974									

							33% of Group 1 patients compared to 6% of Group 2 patients; Incomplete recovery seen in 14% of Group 1 patients compared to 20% of Group 2 patients)		

* Significant Finding

ADM= Admission; ATA= x1 Atmospheric Pressure; $AVDO_2$= Arteriovenous Differences in Oxygen; BAL= Bronchial Alveolar Lavage; CBF= Cerebral Blood Flow; $CMRO_2$= Cerebral Metabolic Rate of O_2; CPP= Cerebral Perfusion Pressure; CSF= Cerebral Spinal Fluid; CT= Computed Tomography; FiO_2= inspired oxygen percentage; GCS= Glasgow Coma Scale; GOS= Glasgow Outcome Scale; HBO= Hyperbaric Oxygen Treatment; hr= Hours; ICP= Intracranial Pressure; IL= Interleukin; ICP= Intracranial Pressure; LPR= Lactate/Pyruvate Ratio; min= Minutes; mmHg= millimeters Mercury; N= sample size; N/A= Not Applicable; NM= Not Mentioned; PbO_2= Brian Tissue Oxygenation; TBI= Traumatic Brain Injury

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Table 5: Clinical Data on HBO followed by NBH for Acute TBI

Phase 2 Data:

Author & Year	NINDS Criteria Ranking	Treatment Groups; N	Eligibility	Blinding and Randomizing Methodology	Adequacy of Controls	Treatment Protocol; Frequency	Positive Treatment Effects	Negative Treatment Effects	Neutral Treatment Effects
Rockswold et al., 2013	6	(1) HBO + NBH; 20 (2) Standard of care; 22	Non-penetrating TBI; PR GCS 3-8 or deterioration to GCS <8 within 48 hours; Marshall Classification ≥2; No prior TBI	Blinded 6 month GOS assessment; Randomized into treatment groups prospectively	Matched for age, gender, ICP, GCS, CT findings, mass lesion evacuations, and decompressive craniectomies	<24 hr post-injury window followed by1 hr of 100% FiO_2 at 1.5 ATA, followed by 3 hr of 100% FiO_2 at 1.0 ATA; 3 consecutive treatments	• Mortality at 6 Months (Group 1 had lower rate of mortality*) • GOS at 6 months (Group 1 had higher rates of favorable outcome*) • PbO_2 (Elevated during treatment compared to		

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| | | | | | | at 24 hr intervals | Group 2*; Pericontusional brain tissue levels remained elevated over Group 2 in post-treatment period*)

• ICP (Reduced levels during treatment compared to Group 2 that was maintained until the next treatment*)

• Dialysate Glycerol (Reduced in | | |

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							Group 1 compared to Group 2*) • Dialysate Lactate (Decreased in injured brain tissue during treatment and into the post-treatment period compared to controls*) • Dialysate LPR (Decreased compared to controls in post-treatment*) • BAL levels of IL-6		

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						and IL-8 did not change • CSF F2-isoprostane did not change		

*Significant Finding

ATA: x1 Atmospheric Pressure; BAL= Bronchial Alveolar Lavage; CSF= Cerebrospinal Fluid; CT= Computed Tomography; FiO_2= inspired oxygen percentage; GCS= Glasgow Coma Scale; GOS= Glasgow Outcome Scale; ICP= Intracranial Pressure; IL= Interleukin; HBO= Hyperbaric Oxygen Treatment; hr= Hours; LPR= Lactate/Pyruvate Ratio; N= sample size; NBH= Normobaric Hyperoxia; PbO_2= Brian Tissue Oxygenation; PR GCS= Postresuscitation GCS; TBI= Traumatic Brain Injury

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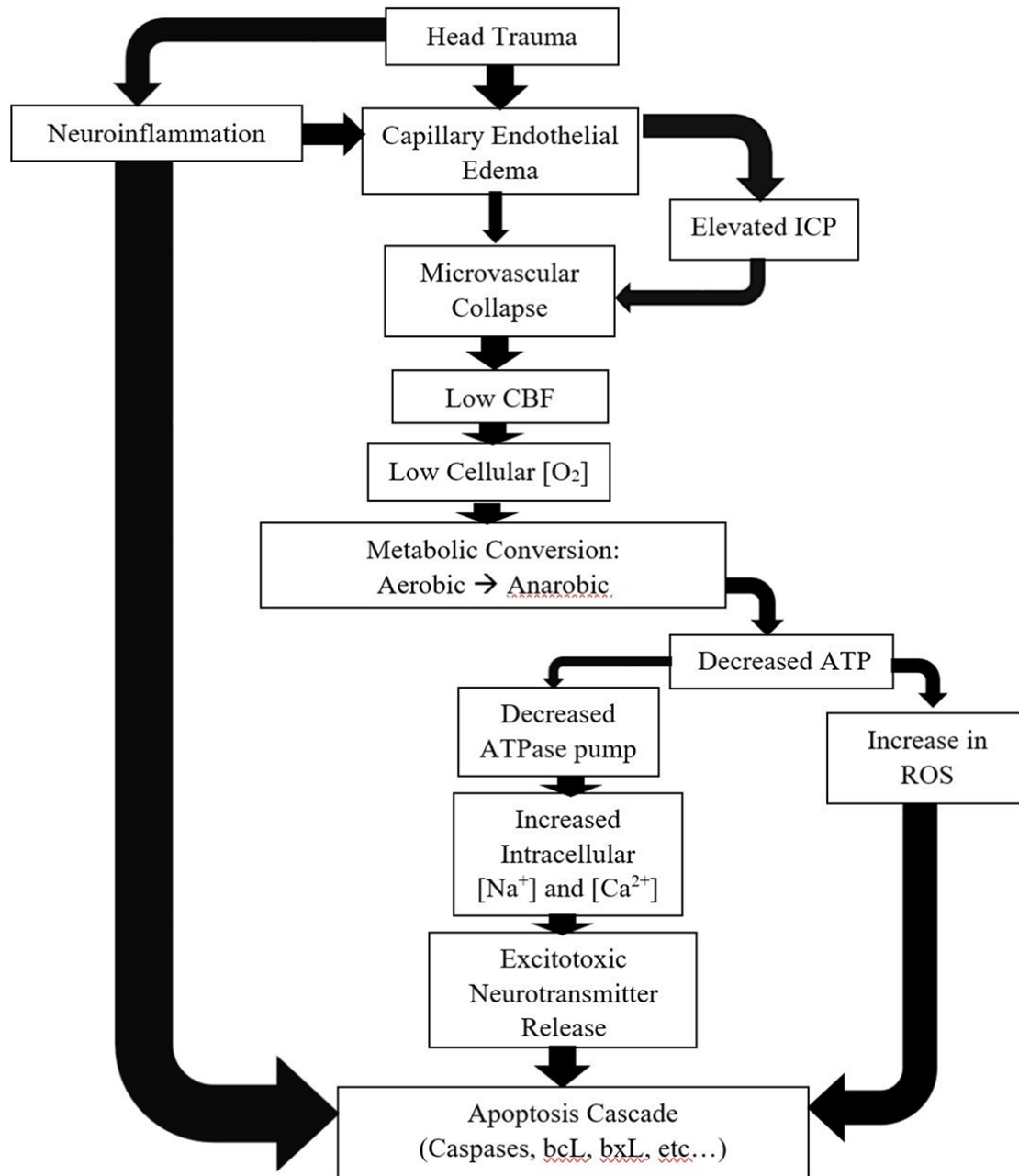
FIGURE LEGENDS

Figure 1. Flow Chart of TBI Pathology

Legend: This figure represents the cellular cascade to apoptosis in the acute phase of a TBI.