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Review of hyperbaric therapy & hyperbaric oxygen therapy in the treatment of neurological disorders according to dose of pressure and hyperoxia

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Review of hyperbaric therapy & hyperbaric oxygen therapy in the treatment of neurological disorders according to dose of pressure and hyperoxia

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Editorial: Review of hyperbaric therapy & hyperbaric oxygen therapy in the treatment of neurological disorders according to dose of pressure and hyperoxia

Paul G. Harch[1,2]*, George Mychaskiw[3], John H. Zhang[4], Dominic P. D'Agostino[5], Keith Van Meter[2] and Enrico M. Camporesi[5]

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KEYWORDS

hyperbaric oxygen therapy (HBOT), hyperbaric therapy, treatment, neurological disorders, pressure, hyperoxia, dosing, brain disorders

Editorial on the Research Topic
Review of hyperbaric therapy & hyperbaric oxygen therapy in the treatment of neurological disorders according to dose of pressure and hyperoxia

Introduction

Hyperbaric therapy (HT) and hyperbaric oxygen therapy (HBOT) have confused the scientific, medical, and lay communities for 362 years since their first use in England by Nathaniel Henshaw (1662). Therapeutic effectiveness has been attributed to increased barometric pressure for the first 300 years and to increased pressure of oxygen in the modern era. A fortuitous observation by the U.S. Food and Drug Administration in 2011 elucidated the contribution of both barometric pressure and hyperoxia and their bioactivity on a continuum of pressure and hyperoxia. Simultaneous research identified epigenetic activity as a primary mechanism of both pressure and hyperoxia. Given these developments and the drug-like effects of HT and HBOT this Research Topic attracted 13 articles that addressed the dosing of pressure and hyperoxia in HT and HBOT for neurological conditions:

Acute and chronic pediatric ischemic/hypoxic neurological injury

Mielecki et al. and Sánchez-Rodríguez and López reviewed the animal and human literature on HBOT for perinatal asphyxia and hypoxic-ischemic encephalopathy,

concluding that HBOT is safe and more effective than hypothermia in ameliorating or eliminating the sequelae of acute global hypoxic/ischemic insult and subsequent HIE with as little as one rescue treatment administered shortly after birth. Doses in animals were generally at 2.0 ATA and above, while doses in humans tended to be under 2.0 ATA. Marois et al. compared the effectiveness of typical therapies for the common chronic form of pediatric ischemic/hypoxic and hemorrhagic brain injury, cerebral palsy, and demonstrated that HBOT is four times as effective as the average of all other therapies. Using a precise analytical tool the authors found that increased barometric pressure (1.3–1.75 ATA) is the dominant contributor to the effect of HBOT compared to hyperoxia. In light of the science of HBOT in acute HIE and chronic neurological injury, the number of children affected, the devastating consequences, and the impact on quality of life these articles reinforce earlier conclusions that "The time has come"…"for HBOT to be standard of care" [SIC] for these conditions (1).

Chronic neurological injury

Slade et al. and Jingami et al. reported the first cases of temporally-demonstrated HBOT benefit in chronic stroke thalamic pain syndrome and subacute delayed post-hypoxic leukoencephalopathy (DPHL) secondary to opioid overdose. Post-stroke pain disorders reduce quality of life and affect mood, sleep, and social function. In Slade et al. the authors treated a 55-year-old man who suffered a right thalamic lacunar infarction and developed thalamic pain syndrome. With extensive HBOT over 11 months (100 treatments) the patient's symptoms resolved and his quality of life improved. Jingami et al. reported on the use of acute high-dose HBOT (2.8 ATA oxygen) for suspected carbon monoxide poisoning in a 47-year-old man who was discovered to have overdosed on opioids. Two months later they re-intervened with a dose of HBOT similar to that used by Slade et al. (2.0 ATA oxygen) when DPHL developed. With 62 HBOTs over 140 days, the patient experienced resolution of the neurological symptoms of his ischemic/hypoxic injury. While HBOT can improve cerebral blood flow and metabolism in chronic brain injury this patient's clinical improvement with HBOT raises interesting questions since the improvement occurred despite worsened fronto-parietal ioamphetamine SPECT imaging and evolving cortical atrophy.

Cancer

Alpuim Costa et al. discussed the use of hyperbaric oxygen therapy (HBOT) as an adjunctive treatment for neuroblastoma, a common pediatric cancer. The authors noted that HBOT reverses tumor hypoxia, a major cause of resistance to traditional treatments such as chemotherapy and radiotherapy, and has other beneficial effects on cancer. Its greatest effect, however, may be modification of the tumor microenvironment (TME) and enhancing the immune response against neuroblastoma cells. Alpuim Costa et al. also reviewed a controlled trial of an HBOT-enhanced IV tumor-specific radioactive compound in children with recurrent Stage IV neuroblastoma, which demonstrated an increase in survival in the HBOT group that was over 2.5 times the control group. The authors recommended future research with HBOT, immunotherapy, and ketone metabolic therapy (KMT) to exploit the unique metabolic vulnerabilities of cancer cells. Wang et al. also discussed the potential of HBOT to enhance cancer immunotherapy through its modification of the TME. HBO enhances radiotherapy and photodynamic therapy by increasing the production of reactive oxygen species and restructuring the extracellular matrix to promote immune cell infiltration. The authors reviewed the effects of HBOT on cancer-specific immune targeting by noting HBOT's ability to improve T cell access to the tumor site, which boosted the effectiveness of immune checkpoint inhibitors such as PD-1/PD-L1 antibodies. Their review of HBOT animal cancer studies showed the effectiveness of HBOT at higher doses in combination with chemotherapy and immunotherapy while HBOT alone had mixed effects on tumor growth.

Pressure—[diving and altitude (dementia)]

Two manuscripts by Fogarty and Harch and MacLaughlin et al. addressed the second key component of HBOT, barometric pressure, through the application of hyperbaric air. Fogarty and Harch recounted a fascinating history of the bioactivity of low barometric pressure fluctuations, beginning with observations by Evangelista Torricelli in 1644, Henshaw's suggestion of pressurized air as a medical therapy in 1662, O.J. Cunningham's application of pressurized air to Spanish influenza patients in 1918, and M.R. Kramer's successful treatment of COPD patients with 0.14 atmospheres of increased pressurized air in 1996. Fogarty and Harch presented the case of an elderly patient with dementia whose functions were affected by travel involving changes in altitude: deterioration with increasing altitude (decreased pressure) and improvement with decreasing altitude (increased pressure). The authors duplicated the increased pressure benefit with a daily 0.3-atmosphere hyperbaric air treatment (and supplemental glutathione amino acid precursors), which generated a sustained improvement in dementia symptoms. MacLaughlin et al. used the same 0.3-atmosphere increase in air to produce an increase in circulating stem progenitor cells in human subjects. Both papers convincingly suggest that the use of low-dose hyperbaric air as a placebo control in randomized, controlled hyperbaric trials is a treatment in itself, not a control. Simonnet et al., addressed a long-standing debate about the greater contribution of pressure vs. oxygen in the treatment of divers with spinal decompression sickness. The authors' experience suggested that a higher oxygen pressure in the initial treatment is more efficacious than a deeper pressure with less oxygen. This is a provocative finding that does not explain the long-standing U.S. Navy treatment algorithm of converting a failing shallow oxygen table to a deeper pressure table. The findings of Simonnet et al.'s, await replication.

Psychiatric disease

Andrews and Harch reported a systematic review of HBOT for PTSD. Statistically significant symptomatic improvements were

achieved over a wide range of pressures from 1.3 to 2.0 ATA with a linear dose-response relationship between improvement and cumulative oxygen dose. This was accompanied by a severe reversible exacerbation of emotional symptoms at the highest oxygen doses in 30%—39% of subjects, a potential effect of oxygen toxicity. The most surprising findings were imaging abnormalities in PTSD-affected brain regions, suggesting that PTSD should no longer be considered a strictly psychiatric disease.

Oxygen toxicity

Zaghloul et al. and Harch and Rhodes studied oxygen toxicity across a spectrum of doses from normobaric to hyperbaric oxygen. Zaghloul et al. demonstrated a protective effect of Galantamine administration on mouse pups living in a normobaric hyperoxic environment. Medullary, forebrain, and hippocampal hyperoxia-induced neuronal loss, behavioral/learning/memory deficits, hyaloid artery hyperplasia, retinal cell disruption, and neovascularization were all reduced by daily galantamine administration. These data are highly suggestive of a neuroprotective and ocular protective role for galantamine in oxygen-dependent neonates. In contrast, Harch and Rhodes reported a 20-year experience of acute and chronic central nervous system oxygen toxicity observed during hyperbaric hyperoxic treatment of chronic neurological conditions. Chronic oxygen toxicity was documented at significantly lower pressures over an often extended number of treatments, as measured by a quantitative parameter of cumulative oxygen dose. Multiple clinical observations of hyperoxia were observed before patients attained the parabolic threshold that is documented in the published literature. One case with functional neuroradiological evidence reinforced the significance of these data.

Conclusion

The above collection of articles has served the purpose of this Research Topic by demonstrating a wide range of bioactivity of hyperbaric pressure and oxygen on disease physiology, pathology, and neurological diseases. The perspective provided will hopefully stimulate additional research into the effects of hyperbaric oxygen and pressure dosage and clinical applications of HBA and HBOT on neurological conditions. It is apparent that after 362 years, the field of hyperbaric medicine is in the infancy of neurological dosing.

Author contributions

PH: Conceptualization, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. GM: Writing – original draft, Writing – review & editing. JZ: Writing – original draft, Writing – review & editing. DD'A: Writing – original draft, Writing – review & editing. KV: Writing – original draft, Writing – review & editing. EC: Writing – original draft, Writing – review & editing.

Conflict of interest

PH is the owner of an S Corporation that is the vehicle for his practice of hyperbaric medicine. He also performs consulting and gives expert witness testimony under this S Corp.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Hyperbaric air mobilizes stem cells in humans; a new perspective on the hormetic dose curve

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Introduction: Hyperbaric air (HBA) was first used pharmaceutically in 1662 to treat lung disease. Extensive use in Europe and North America followed throughout the 19th century to treat pulmonary and neurological disorders. HBA reached its zenith in the early 20th century when cyanotic, moribund "Spanish flu pandemic" patients turned normal color and regained consciousness within minutes after HBA treatment. Since that time the 78% Nitrogen fraction in HBA has been completely displaced by 100% oxygen to create the modern pharmaceutical hyperbaric oxygen therapy (HBOT), a powerful treatment that is FDA approved for multiple indications. Current belief purports oxygen as the active element mobilizing stem progenitor cells (SPCs) in HBOT, but hyperbaric air, which increases tensions of both oxygen and nitrogen, has been untested until now. In this study we test HBA for SPC mobilization, cytokine and chemokine expression, and complete blood count.

Methods: Ten 34–35-year-old healthy volunteers were exposed to 1.27ATA (4 psig/965 mmHg) room air for 90 min, M-F, for 10 exposures over 2-weeks. Venous blood samples were taken: (1) prior to the first exposure (served as the control for each subject), (2) directly after the first exposure (to measure the acute effect), (3) immediately prior to the ninth exposure (to measure the chronic effect), and (4) 3days after the completion of tenth/final exposure (to assess durability). SPCs were gated by blinded scientists using Flow Cytometry.

Results: SPCs ($CD45^{dim}/CD34^{+}/CD133^{-}$) were mobilized by nearly two-fold following 9 exposures (p=0.02) increasing to three-fold 72-h post completion of the final (10th) exposure (p=0.008) confirming durability.

Discussion: This research demonstrates that SPCs are mobilized, and cytokines are modulated by hyperbaric air. HBA likely is a therapeutic treatment. Previously published research using HBA placebos should be re-evaluated to reflect a dose treatment finding rather than finding a placebo effect. Our findings of SPC mobilization by HBA support further investigation into hyperbaric air as a pharmaceutical/therapy.

KEYWORDS

$CD34^{+}$, Traumatic Brain Injury, CD133, war veteran self harm, war veterans' psychological suffering, war veterans suicide, hyperbaric air placebo, HBOT

Introduction

Presently, hyperbaric air (HBA) is not thought of as a medication. Its singular approved medicinal use is for Acute Mountain Sickness. Historically it has been used medicinally, initially reported by Henshaw in 1662 to treat "afflictions of the lungs" (1) and additional reports from

TABLE 1 Anthropometric data of subjects in this hyperbaric air study.

	N	Mean	Standard deviation
Age	10	34.54 years	1.36 years
Height	10	171.75 cm	9.38 cm
Weight	10	81.40 kg	21.05 kg
BMI	10	27.43%	2.8%
Female	5	5	NA

Europe and America followed (1). In 1857 Simpson published a paper using HBA to treat lung pathologies including tuberculosis (2). Interest in hyperbaric air as a medication surged following successful treatments of "Spanish flu" patients by Cunningham in 1918 (3–5). Unfortunately, Cunningham produced a paucity of papers supporting his work and when he died in 1937, interest in hyperbaric air abated.

HBA began to be employed again in the modern era with controversial results. It was used as a placebo by Collet while investigating the use of hyperbaric oxygen in Cerebral Palsy (6) and also as a placebo in brain injury by Miller, Wolf and Cifu (7–9). In each of these experiments, both the treatment group and the placebo group improved revealing an apparent placebo effect. The use of hyperbaric air as a placebo is energetically debated and controversial because it increases the oxygen tension. In contrast to the placebo findings, similar published studies not using the hyperbaric air placebo, found significant improvements in brain injury (10–20).

Considering the aforementioned, we asked the question, "is hyperbaric air an appropriate placebo?". We searched the literature and found no evidence that hyperbaric air has been tested. We designed a test of hyperbaric air using a gold standard endpoint of oxygen therapy, stem cell mobilization.

Our specific aims were (1) to characterize stem cell mobilization in healthy adults following daily exposures to hyperbaric air, (2) determine if other biomarkers were modulated, (3) determine if there were acute changes, and (4) if so, were the changes durable. Based on previous research done in our lab (21) and calculating a 27% increase in oxygen partial pressures in the inhaled gases, we hypothesized that (1) stem cells would be mobilized, (2) biomarkers would be modulated (3) there would be acute changes and (4) the stem cell mobilization would be durable.

Materials and methods

Design and subjects

This prospective hyperbaric air study was a randomized, single-blind study conducted at the University of Wisconsin – Madison Clinical Sciences Center between May 1st, 2021, and August 31st, 2021. This study is approved by the Institutional Review Board of the University of Wisconsin – Madison. UW IRB ID: 2020-0293-CR001. All participants provided written informed consent. Healthy adults were recruited for participation in this study (Table 1).

Hyperbaric air exposure

All exposures were at 1.27 ATA (4 psig) of room air for 90 min in a Gamow style Mountain Sickness Chamber (Hyperbaric Technologies Inc. Amsterdam, NY, United States). To minimize circadian cell cycle variations including acrophase and circannual cycle, all subjects were diurnally active and all hyperbaric exposures and sample collections occurred at the same time of day over the contiguous 15-day experiment.

Sample collection

Each peripheral venous blood sample was collected during the subject's exposure time slot using either a 21- or 23-gauge BD Vacutainer Safety-Lok Blood Collection Set (Becton, Dickinson and Company, Franklin Lakes, NJ, United States) into a Cyto-Chex BCT tube (Streck Inc. NE USA), BD Vacutainer Plastic Blood Collection Tubes with K2EDTA, BD Vacutainer Plastic Blood Collection Tubes – PST Plasma Separation Tubes, and Greiner Bio-One K2EDTA GelTubes. All samples were stored according to manufacturer's directions. Study protocol in graphic format is included in Figure 1.

Flow cytometry

Fluorescence minus one (FMO) tubes, rainbow bead tubes, and single antibody tubes were prepared as gating references. Antibodies were pipetted into flow cytometry tubes according to manufacturer's instructions. Antibodies used include CD34 = Brilliant Violet 421 (BioLegend, San Diego, CA, United States), CD45 = Alexa Fluor 488 (BioLegend, San Diego, CA, United States), CD133 = PE (Miltenyi Biotec, North Rhine-Westphalia, Germany) CD31 = Brilliant Violet 605 (BioLegend, San Diego, CA, United States), CD105 = PE-Cy7 (BioLegend, San Diego, CA, United States), Ghost Dye Red 780 = Tonbo Biosciences, San Diego, CA.

Flow cytometry was performed by blinded scientists on a ThermoFisher Attune NxT (Waltham, MA, United States). Samples were analyzed by blinded scientists using FlowJo software (FlowJo, Ashland, OR, United States).

Enzyme-linked immunosorbent assay

The Invitrogen ProcartaPlex™ Human Immune Monitoring Panel 65-Plex (Invitrogen, Waltham, MA, United States) was used to assess changes in cytokines, chemokines and growth factors. All tests were performed by blinded scientists at the University of Wisconsin Non-Human Primate Research Center (Madison, WI, United States).

Statistical analysis

The first blood draw taken prior to the first exposure served as the control. To determine whether there was an overall effect across exposures, we utilized the Friedman test (nonparametric alternative to one-way ANVOA with repeated measures) and if significant, comparisons between all-time points were performed using the Wilcoxon signed rank test. Significance level was determined *a priori* at the 0.05 level and all tests were two-tailed. Statistical analyses were calculated using Graph Pad Prism (GraphPad Prism 9.0.0 Software, San Diego, CA, United States).

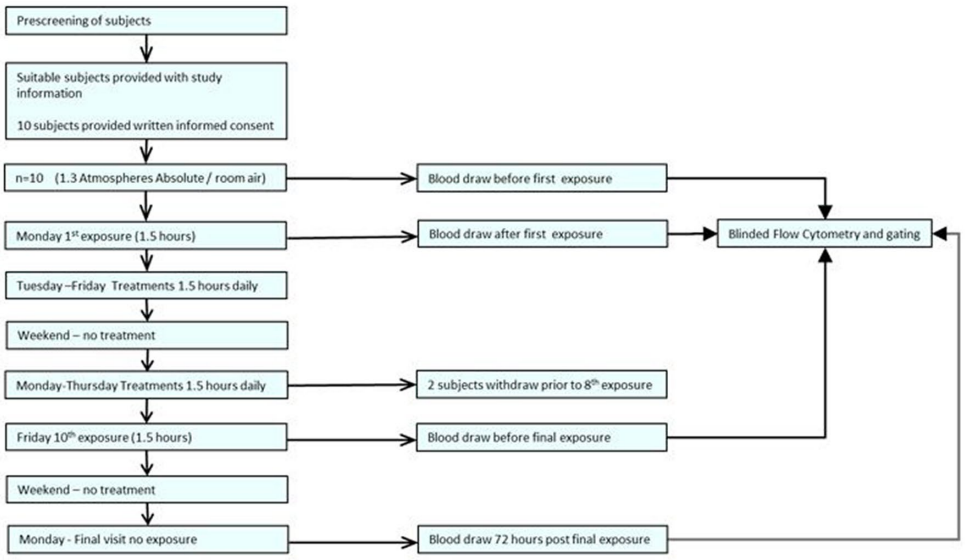

FIGURE 1
Experimental protocol.

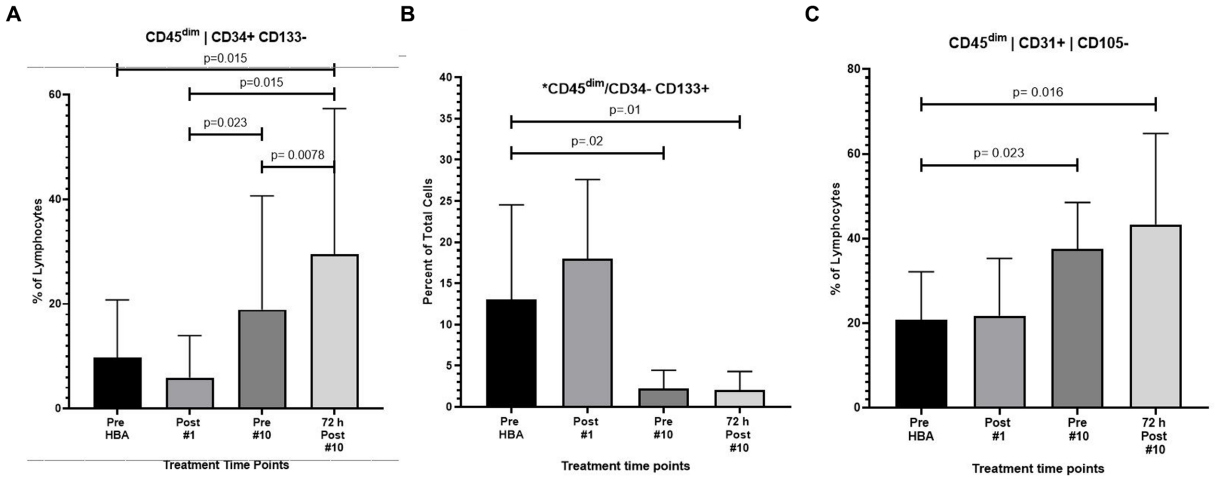

FIGURE 2
Frequency of $CD45^{dim}/CD34^{+}/CD133^{-}$, $CD45^{dim}/CD34^{-}/CD133^{+}$ and $CD45^{dim}/CD31^{+}/CD105^{-}$ after intermittent hyperbaric air exposure detected by flow cytometry. **(A)** $CD34^{+}$ and $CD133^{-}$ stem progenitor cells. **(B)** $CD34^{-}$ and $CD133^{+}$ stem progenitor cells. **(C)** $CD31^{+}$ and $CD105^{-}$ stem progenitor cells.

Results

$CD45^{dim}$

Increased frequency of $CD45^{dim}/CD34^{+}/CD133^{-}$ following nominal exposure to hyperbaric room air

As previously described 10 humans were exposed to 1.27 ATA of room air 10 times over the course of a 12-day period. Results revealed a significant increase in the frequency of $CD45^{dim}/CD34^{+}/CD133^{-}$ stem progenitor cells in venous blood resulting in an approximate two-fold increase directly prior to the 10th exposure ($p = 0.02$). $CD45^{dim}/CD34^{+}/CD133^{-}$ SPCs continued to increase during the 3 days following the end of exposures and increased to three-fold, 72 h after the 10th exposure ($p = 0.008$); (Figure 2A).

Decreased frequency of $CD45^{dim}/CD34^{-}/CD133^{+}$ after hyperbaric exposure

While $CD45^{dim}/CD34^{+}/CD133^{-}$ SPCs increased, the frequency of $CD45^{dim}/CD34^{-}/CD133^{+}$ primitive pro-angiogenic stem progenitor cells significantly decreased after exposure to intermittent hyperbaric air. $CD45^{dim}/CD34^{-}/CD133^{+}$ decreased by nearly five-fold prior to the 10th exposure ($p = 0.02$) and mobilization decreased by six-fold 72 h after the 10th exposure ($p = 0.01$) (Figure 2B).

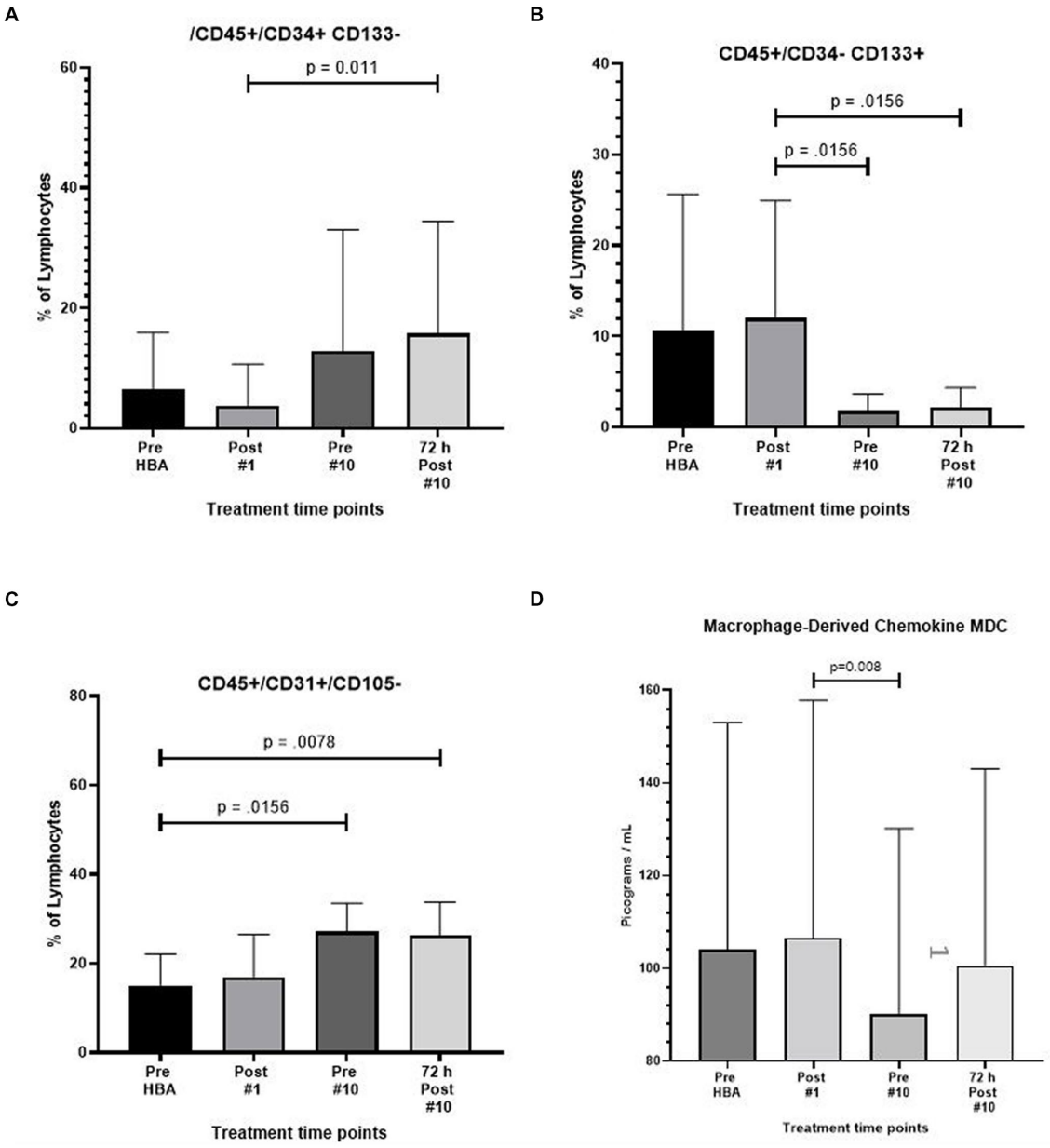

FIGURE 3
Frequency of CD45$^+$/CD34$^+$/CD133$^-$, CD45$^+$/CD34$^-$/CD133$^+$, CD45$^+$/CD31$^+$/CD105$^-$, and macrophage derived chemokine after intermittent hyperbaric air exposure detected by flow cytometry and ELISA. **(A)** CD34$^+$ and CD133$^-$ stem progenitor cells. **(B)** CD34$^-$ and CD133$^+$ stem progenitor cells. **(C)** CD31$^+$ and CD105$^-$ stem progenitor cells. **(D)** Macrophage derived chemokine.

Increased frequency of CD45^{dim}/CD31$^+$/CD105$^-$ after hyperbaric exposure

The frequency of CD45dim/CD31$^+$/CD105$^-$ was also significantly increased after hyperbaric exposure by nearly two-fold prior to the 10th exposure ($p = 0.023$) and increased to over two-fold 3 days after the 10th exposure ($p = 0.016$) (Figure 2C).

Mobilization of CD45$^+$/CD34$^+$/CD133$^-$

The expression of CD45$^+$/CD34$^+$/CD133$^-$ also increased by nearly 3.5 fold after 9 full exposures ($p = 0.012$) and increased to over four fold 72 h following the end of the 10th exposure ($p = 0.002$) (Figure 3A).

Decreased frequency of CD45$^+$/CD34$^-$/CD133$^+$

A significant reduction in the expression of CD45$^+$/CD34$^-$/CD133$^+$ was noted resulting in a six-fold reduction prior to the 10th exposure ($p = 0.016$) and recovering to 5.4-fold 72 h after the 10th and final exposure ($p = 0.016$) (Figure 3B).

TABLE 2 Summary of cell type frequency after initial and intermittent exposure detected by flow cytometry.

	$CD45^{dim}$	$CD45^{+}$	$CD45^{-}$
$CD45^{dim}$	=,=,=,=		
$CD45^{+}$		=,=,=,=	
$CD45^{-}$			=,=,↓,=
$CD31^{-}$ $CD105^{+}$	=,=,=,=	=,=,=,=	=,=,=,=
$CD31^{+}$ $CD105^{-}$	=,=,↑,↑	=,=,↓,↓	=,=,=,=
$CD31^{+}$ $CD105^{+}$	=,=,=,=	=,=,=,=	=,=,=,=
$CD31^{-}$ $CD105^{-}$	=,=,=,=	=,=,=,=	=,=,=,=
$CD34^{-}$ $CD133^{+}$	=,=,↓,↓	=,=,↓,↓	=,=,=,=
$CD34^{+}$ $CD133^{-}$	=,=,↑,↑	=,=,↑,↑	=,=,=,=
$CD34^{+}$ $CD133^{+}$	=,=,=,=	=,=,=,=	=,=,=,=
$CD34^{-}$ $CD133^{-}$	=,=,=,=	=,=,=,=	=,=,=,=

Results from four time points represented by change symbol separated by commas. The = symbol represents no change in frequency, the ↓ symbol represents a significant reduction in frequency, and the ↑ symbol indicates a significant mobilization. The first time point is the control point taken prior to exposure. The second is taken directly after the first exposure. The third timepoint was taken just prior to the 10th and final exposure and the fourth time point is the frequency 3 days after cessation of exposures.

Mobilization of $CD45^{+}/CD31^{+}/CD105^{-}$

The expression of $CD45^{+}/CD31^{+}/105^{-}$ increased by nearly two-fold after 9 full exposures ($p = 0.02$) and remained at the level through 72 h following the end of the 10th exposure ($p = 0.008$) (Figure 3C).

Complete results from flow cytometry

Complete results from flow cytometry testing, including non-significant findings are included in tabular form for reference. (Table 2).

Macrophage-derived chemokine (MDC) expression significantly decreased

Results from the Invitrogen ProcartaPlex™ Human Immune Monitoring Panel 65-Plex ELISA like analysis showed there was only one change to report. The expression of Macrophage-derived Chemokine (MDC) was significantly lower between the second and third time points ($p = 0.008$). All other tests revealed no change (Figure 3D).

Complete blood count with differential

There were no significant changes in CBC at any time points (Table 2).

Discussion

The smallest dose of hyperbaric air that will result in a therapeutic effect is unknown and strongly debated among scientists and physicians. Pressures below 1.4 atmospheres absolute of hyperbaric air are accepted as a placebo but have not previously been tested in humans. Studies using hyperbaric air as a placebo have resulted in a "placebo" or "participation" effect (7–9). This placebo effect finding is vigorously disputed because hyperbaric air significantly increases the partial pressure of oxygen (and nitrogen) in the inspired air (22–27). The "placebo effect" findings have effectively restricted the use of HBOT for many conditions including Traumatic Brain Injury in soldiers returning from combat.

In this study we asked the question, will a small dose of hyperbaric air (1.27 ATA), that is below the accepted 1.4 ATA therapeutic threshold, mobilize stem cells similar to HBOT? We hypothesized that stem cells would be mobilized.

Indeed, stem cells were significantly mobilized, refuting previous "placebo effect" findings. We intend that the results will provide needed experimental data to medical societies and journal editors, and in turn provide guidance to FDA and physicians.

Critical analysis of major findings

Testing for stem cell mobilization using flow cytometry and gated by blinded scientists, the major finding of this study is that 1.27 ATA hyperbaric air mobilizes $CD34^{+}/CD133^{-}$ SPCs and $CD31^{+}/CD105^{-}$ stem cells in humans receiving 10 daily 90-min exposures (Figures 2A,C, respectively). Mobilization of $CD34^{+}$ SPCs has also been observed in isobaric hyperoxic (21) and in hyperbaric hyperoxic conditions (28, 29).

The mechanism of hyperbaric air SPC mobilization in this experiment is beyond the scope of this study, but may be similar to SPC mobilization found in hyperbaric oxygen therapy, which activates nitric oxide synthase and plays a prime role in initiating $CD34^{+}$ SPC mobilization (30–33). ($CD34^{+}$ background) $CD34^{+}$ adult stem/progenitor cells are a group of specific cell types that possess the abilities of self-renewal and multipotent differentiation (34, 35).

$CD34^{+}$ is expressed on hematopoietic and pro-angiogenic stem progenitor cells and on endothelium (36). Pro-angiogenic stem/progenitor cells contribute to neovascularization by a process of homing to ischemic tissue called vasculogenesis, and by budding endothelium from established blood vessels in a process called angiogenesis (37, 38). Further research is needed to understand the homing and function of the $CD34^{+}$ SPCs mobilized by intermittent hyperbaric air exposures.

A second significant finding of this study, which we report for the first time, is hyperbaric air mobilizes $CD31^{+}/CD105^{-}$ SPCs. This novel finding has many implications in the field of hyperbaric air and hyperbaric oxygen. ($CD31^{+}$ background) CD31 is thought to have a protective role in experimental atherosclerosis (39). The therapeutic potential of CD31 agonists to manage atherosclerotic disease manifestations is a consistent finding in pre-clinical studies (40). Although this is the first time that CD31 has been reported to be mobilized by either hyperbaric air or hyperbaric oxygen, it is not unexpected, given that CD31 is associated with endothelial function much like CD34 and CD133. It was beyond the scope of this research project to determine if these mobilized CD31 stem cells participate in wound healing and angiogenesis. Future research testing the effect of hyperbaric air mobilized CD31 stem cells on healing, angiogenesis, and atherosclerotic disease states would be prudent.

Our results also revealed an interesting relationship between SPCs expressing $CD45^{dim}/CD34^{+}/CD133^{-}$ and those SPCs expressing $CD45^{dim}/$

$CD34^{-}/CD133^{+}$. CD133+ SPCs are hematopoietic precursors to $CD34^{+}$ and almost all hematopoietic pluripotent and committed stem cells in colony-forming assays express $CD34^{+}$ (36). In this study we found that $CD34^{+}$ SPCs increased while at the same time $CD133^{+}$ SPCs decreased. We hypothesize that the exposure to intermittent hyperbaric air may mobilize $CD133^{+}$ from bone marrow, and also play a role in the differentiation of the $CD133^{+}$ primitive hematopoietic precursor into the $CD34^{+}$ SPC. However, it has also been shown that $CD133^{+}$ have a high capacity to differentiate into other cell types including fibroblasts, hepatocytes, and neural cell-types (35). Unfolding the explanation for the significant reduction in the expression of $CD45^{dim}/CD34^{-}/CD133^{+}$ expressing SPCs holds exciting potential.

Previous research has shown that breathing 100% oxygen at 2.4 atmospheres absolute mobilized stem/progenitor cells at a significantly greater rate than 2.0 Atmospheres Absolute suggesting a dose effect (41). HBOT at 2.0 ATA (P_IO^2 of 1426 mmHg and P_IN^2 of 0 mmHg) resulted in a two-fold mobilization of $CD34^{+}$ SPCs after 1 exposure and an eight-fold increase after 20 exposures (28). In this study using 1.27 ATA of hyperbaric air (P_IO^2 of 189 mmHg and P_IN^2 of 706 mmHg) we saw a two-fold increase in SPC mobilization just prior to the 9th exposure. This supports the hypothetical relationship between oxygen dose and stem cell mobilization.

Another aim of this study was to determine if the stem progenitor cell mobilization was durable. Because the stem progenitor cell mobilization increased another fold following the end of exposures a durable effect is likely.

Interestingly, we also found changes in cells expressing $CD45^{+}$ cell subtypes. These changes were remarkably similar to changes in the CD45dim population. $CD45^{+}$ is expressed on all hematopoietic cells, including HSCs and osteoclasts, which are of hematopoietic origin (42), and is known as a pan-leukocyte marker.

Macrophage-derived chemokine (MDC) expressed in venous blood significantly decreased prior to the ninth exposure and returned to pre-exposure levels 72 h following the final exposure. The expression of MDC is increased in idiopathic pulmonary fibrosis (43) and elevated following resuscitation of patients after hemorrhage. Macrophage-derived chemokine may provide a therapeutic strategy to mitigate this inflammatory response (44). MDC is also thought to serve as a marker of pharmacological therapy response in Major Depressive Disorder (45).

Changes in barometric and hydrostatic pressure may be a mechanism of hyperbaric therapy. Small changes in atmospheric pressure elicit responses in many organisms (46). However the mechanism(s) is poorly understood. One possibility is that pressure is sensed through hydrostatic compression of heterogeneous structures. For instance, cells in suspension, including platelets (47, 48) and cartilage cells (49, 50), respond to small changes in pressure ostensibly through this mechanism. Another possibility is that cellular structures may produce shear and strain through differential compression. Microtubules, actin and other cytoskeletal proteins respond to this type of local mechanical stresses (51, 52). It should also be noted that certain animals appear to respond to changes in atmospheric (air) pressure. For example, pigeons exhibit changes in heart rate in response to changes of about 1 mbar, which is a typical daily fluctuation in barometric pressure. But in such cases, it is unclear how barometric pressure changes are sensed (53). It is probable that changes in pressure have effects on human physiology.

Another possible mechanism ultimately resulting in stem cell mobilization in this experiment is a progressive accumulation of endogenous anti-oxidants at the cellular level. These antioxidants accrue in response to repeated exposure to a hyperoxic environment. The result is an up-regulation of the Hypoxia-inducible factor 1α (HIF-1α) transcription factor activity (54–60). This hypothetical mechanism is best described as the Normoxic Hypoxic Paradox. This mechanism is best characterized by an increase in reactive oxygen species (ROS) due to the hyperoxic cellular environment. The increased cellular ROS creates an imbalance of ROS/scavenger antioxidants ratio. The increased ROS molecules initially hydroxylate most of the HIF-1α, facilitating ubiquitination and degradation of most of the HIF-1α subunits. However, it is postulated that an adaptive response to repeated hyperoxia, increases the production of scavengers in proportion to the increased ROS generation. The ROS/scavenger ratio gradually becomes balanced in the hyperoxic environment. However, when the hyperoxic exposure ends and the cell returns to a normoxic state, the ROS/scavenger ratio is again imbalanced, but this time with more scavengers than ROS, which produces a reduced ROS environment. Less ROS leads to an increase in HIF-1α, initiating HIF transcription factor activity, resembling a hypoxic state, but in a normoxic environment. In this study $CD34^{+}/CD133^{-}$ expression trended downward directly following the initial hyperbaric air exposure, but increased significantly prior to the tenth exposure. The opposite was true with $CD34^{-}/CD133^{+}$. These results support the hypothetical Normoxic Hypoxic Paradox mechanism.

While our study clearly showed that the hyperbaric air dose that was used as a placebo mobilizes proangiogenic and hematopoietic stem progenitor cells and likely has a therapeutic physiologic effect similar to hyperbaric oxygen therapy (29, 61–63), it was beyond the scope of this study to determine clinical significance. Hopefully our results will generate renewed interest in hyperbaric air and future studies will investigate both mechanism and clinical outcomes.

Limitations

The major limitation of our study is a relatively small sample size.

Future directions

It should not be overlooked that although we increased the oxygen partial pressure and presume that oxygen is the active element in hematopoietic and pro-angiogenic stem progenitor mobilization, the partial pressure of nitrogen and remaining trace gasses are also increased. Nitrogen is not an inert element and can form five potential oxidation states and three potential reduction states with various levels of reactivity (64). Recent evidence suggests that intracellular reactive oxygen and nitrogen species play an important role in intracellular signaling cascades (65). However, little is known about the effect of nitrogen and trace gasses on SPC mobilization or cytokine, chemokine and growth factor modulation. Increased nitrogen and trace gasses as a stimulator of SPC mobilization and chemokine modulation is a possible mechanism in this research project and an exciting idea to explore in future research.

It is very possible that stem cell mobilization by hyperbaric air provides an additional modality to heal injuries. Because of its reduced cost of delivery, it may prove beneficial in developing nations and in underserved populations. Hyperbaric air also increases safety because the oxygen partial pressure is relatively low. The low oxygen partial

pressure in hyperbaric air may also provide increased therapeutic value by not overloading the cerebral energy metabolism balance (66, 67).

Finally, our data suggests that the therapeutic dose of oxygen begins at a much smaller partial pressure than previously thought and adds a new data point on the initial portion of the hormetic curve of oxygen dose.

Although we found that intermittent small increases in hyperbaric air pressure mobilized stem cells, this study should not be taken as an endorsement of the use of intermittent hyperbaric air for any purpose other than the indications approved by the FDA.

Why are these findings important? First, prior to this research it was not known that breathing a small increase in hyperbaric air would mobilize stem progenitor cells. This knowledge could be an important low-cost healthcare option when hyperbaric oxygen is not available, especially in underserved populations, remote areas, and developing nations. Second, because the technology is lightweight and portable, it is hypothetically possible to be used when transporting combatants and civilians out of a warzone to extend the viability of damaged tissue and to reduce exacerbating gas embolism when air-lifting by high altitude flights is required. Finally, this research refutes the findings of a placebo effect in the decades-old use of slightly pressurized room air as a placebo in hyperbaric oxygen research.

Conclusion and impact

In this study we demonstrate for the first time that intermittent exposure to ostensibly insignificant pressures of hyperbaric air mobilizes stem progenitor cells in a similar manner to that seen in isobaric hyperoxia and hyperbaric oxygen therapy (21, 28, 29, 41). We also establish that the stem progenitor cell mobilization is durable.

This research reveals that hyperbaric air, even at an ostensibly insignificant dose, has significant effects on human physiology, is not a placebo, and should be considered as an active physiologic intervention. Its use as a pharmaceutical should be investigated further.

Although this study did not test for clinical results, clinical outcomes of hyperbaric air have been reported in similar hyperbaric air studies (6–8). This study supports the data, but refutes the conclusions in those studies by revealing a mechanism of action for the clinical improvements reported in the hyperbaric air group in those studies. Much more work is needed to develop protocols of hyperbaric air dose that provide the maximum therapeutic benefit.

These findings substantiate the need for testing hyperbaric air doses prior to using hyperbaric air as a placebo in scientific investigations. These findings also substantiate the urgent need for reevaluation of findings in historical studies using hyperbaric air placebos. Our findings suggest that these historical placebo-controlled studies were not placebo-controlled studies. Paradoxically, our findings indicate that they were dose studies and the findings of a "placebo effect" or "participation effect" are inherently flawed. The "findings" and "conclusions" in studies using hyperbaric air as a placebo should be reevaluated from a dose study perspective. Finally, we hope the findings in this study will persuade the medical societies around the world to consider reevaluation of their definition of hyperbaric medicine to include nominal hyperbaric air.

Looking back at the "Hyperbaric Air" work of Henshaw, Simpson and Cunningham, our findings support their reports.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the University of Wisconsin–Madison. The patients/participants provided their written informed consent to participate in this study.

Author contributions

KM and GB: ideas conception. KM, GB, RB, and ME: study and experiments design. KM, JM, RB, JL, and MM: experiments perform, data acquisition, and analysis. GB and RB: guidance and critical feedback on data acquisition and analysis. RB and ME: project supervision. KM: manuscript writing. All authors provided critical feedback and contributed to the final version of manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Galantamine ameliorates hyperoxia-induced brain injury in neonatal mice

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Introduction: Prolonged oxygen therapy in preterm infants often leads to cognitive impairment. Hyperoxia leads to excess free radical production with subsequent neuroinflammation, astrogliosis, microgliosis and apoptosis. We hypothesized that Galantamine, an acetyl choline esterase inhibitor and an FDA approved treatment of Alzheimer's disease, will reduce hyperoxic brain injury in neonatal mice and will improve learning and memory.

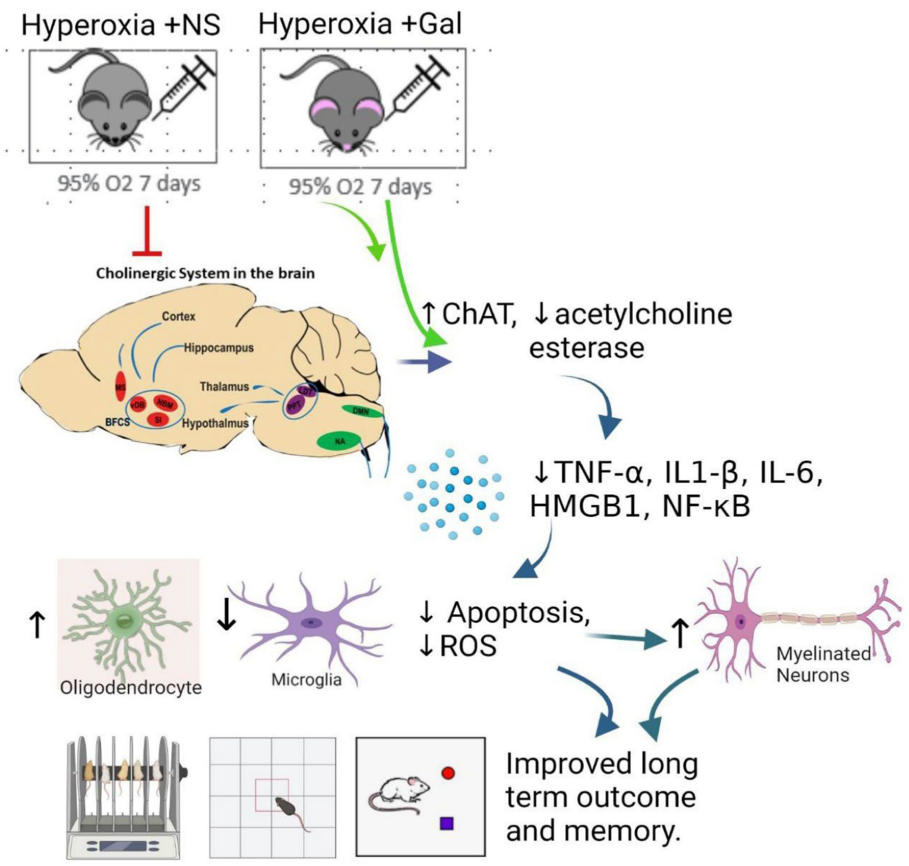
Methods: Mouse pups at postnatal day 1 (P1) were placed in a hyperoxia chamber (FiO_2 95%) for 7days. Pups were injected IP daily with Galantamine (5mg/kg/dose) or saline for 7days.

Results: Hyperoxia caused significant neurodegeneration in cholinergic nuclei of the basal forebrain cholinergic system (BFCS), laterodorsal tegmental (LDT) nucleus and nucleus ambiguus (NA). Galantamine ameliorated this neuronal loss. Treated hyperoxic group showed a significant increase of choline acetyl transferase (ChAT) expression and a decrease of acetyl choline esterase activity, thus increasing acetyl choline levels in hyperoxia environment. Hyperoxia increased pro-inflammatory cytokines namely IL -1β, IL-6 and TNF α, HMGB1, NF-κB activation. Galantamine showed its potent anti- inflammatory effect, by blunting cytokines surges among treated group. Treatment with Galantamine increased myelination while reducing apoptosis, microgliosis, astrogliosis and ROS production. Long term neurobehavioral outcomes at P60 showed improved locomotor activity, coordination, learning and memory, along with increased hippocampal volumes on MRI with Galantamine treated versus non treated hyperoxia group.

Conclusion: Together our findings suggest a potential therapeutic role for Galantamine in attenuating hyperoxia-induced brain injury.

KEYWORDS

hyperoxia induced brain injury, galantamine, acetylcholine esterase inhibitor, neuroinflammation, reactive oxygen species



GRAPHICAL ABSTRACT
Galantamine anti-inflammatory and antioxidant benefits for neonatal hyperoxic brain injury.

Introduction

Brain injury in preterm infants is the leading cause of disability and death in children. Fetal development occurs under relative hypoxic conditions (PaO_2: 25 mmHg) compared to extrauterine life (PaO_2: 70 mm Hg) (Castillo et al., 2008). Premature infants' tissues, including the brain, is exposed to relative hyperoxia compared to the intrauterine environment. This hyperoxia stress is further intensified by O_2 supplementation used in treatment of the respiratory distress syndrome caused by lung immaturity (Felderhoff-Mueser et al., 2004).

In preterm infants, fluctuating or prolonged exposure to supraphysiological O_2 levels may cause disruption of the neuronal circuitry resulting in encephalopathy of prematurity characterized by white matter injury (WMI) (van Tilborg et al., 2016). Furthermore, hyperoxia is one of the key risk factors for disabling cerebral palsy (Collins et al., 2001). Behavioral studies of very low birth weight premature infants exposed to hyperoxia show a heightened risk for anxiety, depression, and autistic like behaviors (Burnett et al., 2011; Pyhälä et al., 2014).

Exposure of neonate mice pups to 95% oxygen for 7 days is a well approved model of bronchopulmonary dysplasia with lung inflammation and impaired alveolarization (Auten et al., 2009). In rodents, hyperoxia causes neurocognitive impairment and memory deficits with smaller hippocampal sizes, similar to brain findings in ex-preterm infants (Ramani et al., 2013). Hyperoxia in rodents caused neuronal death, apoptosis, autophagy, oxidative stress, inflammation, altered neurotrophin growth factors and gene expression related to synaptic plasticity (Kaindl et al., 2006; Yiş et al., 2008; Ikonomidou and Kaindl, 2011; Reich et al., 2016). Rodents exposed to hyperoxia early in life developed hyperactivity and coordination deficits in adolescence, with cognitive impairment persisting into adulthood (Schmitz et al., 2012; Hoeber et al., 2016; Serdar et al., 2016).

Neonates have immature central nervous system with high metabolic demand. Their antioxidant defenses are immature leading to increase accumulation of free radicals (Perrone et al., 2015, 2016). Reactive oxygen species (ROS) inactivate enzymes, destroy DNA and proteins, eventually disrupting membrane function. ROS activates NF-κB leading to neuronal apoptosis and necrosis (Leviton et al., 2015). Free radicals induce mitochondrial membrane damage causing them to become leaky, releasing cytochrome C into the cytoplasm thus activating caspases which lead to severe injury to the developing brain (Love, 1999; Yuan and Yankner, 2000; Filomeni et al., 2003). Oxidative stress causes reactive astrogliosis, microgliosis and inflammation. Augmenting the antioxidant system by the over-expression of superoxide dismutase reduced hyperoxic brain injury in a neonate mouse model (Zaghloul et al., 2012). Since the use of O_2 therapy in the neonatal period cannot be avoided, effective therapies to decrease the deleterious effect of hyperoxia are urgently needed.

Galantamine, a centrally acting acetylcholinesterase inhibitor, is widely used to treat cognitive deficits in Alzheimer's diseases (Bullock, 2004). Galantamine crosses the blood brain barrier, stimulates cholinergic signaling and remains for an extended period of time

(Noetzli and Eap, 2013). The brain cholinergic system plays a major regulatory role in memory and attention (Ballinger et al., 2016). Brain cholinergic signaling controls inflammation via the cholinergic anti-inflammatory pathway. This pathway controls cytokine production and inflammation through the efferent vagus nerve (Gallowitsch-Puerta and Pavlov, 2007). Impaired cholinergic signaling in murine sepsis survivors is a result of the interplay between systemic inflammation and neuroinflammation (Zaghloul et al., 2017). Galantamine minimizes oxidative damage in lymphocytes, using an *in vitro* model (Triana-Vidal and Carvajal-Varona, 2013). Galantamine decreased hypoxia-ischemia brain injury in a newborn rodent model (Furukawa et al., 2014). Physostigmine and donepezil, both cholinesterase inhibitors, reduced the deleterious neuronal effects of 80% oxygen exposure to 6-day old rat pups (Sifringer et al., 2013). However, the effects of Galantamine on hyperoxia induced brain injury has not been studied. In this work we investigate the neuroprotective role of galantamine treatment in neonatal mice who developed brain injury induced by hyperoxia exposure.

Materials and methods

Animal hyperoxia model

All procedures were performed in accordance with the NIH Guidelines on the care and use of vertebrate animals and approved by the Institutional Animal Care and Use Committee of the Feinstein Institute for Medical Research and the University of Arizona. Neonate C57BL6 mice at postnatal day 1 (P1) were placed in hyperoxia chamber system (BioSpherix, Lacona, NY, USA) which provided 95% normobaric oxygen for 7 days. Nursing mothers were switched every 24 h so that they are healthy and can lactate the pups. Animals were divided into 4 groups: room air (RA) + saline, RA + Galantamine, hyperoxia + saline, hyperoxia + Galantamine. Neonate mice were injected intraperitoneally with Galantamine at a dose of 5 mg/kg/day (dissolved in saline) at a volume of 0.1 mL. They were injected daily for 7 days starting from P1 after being placed in the hyperoxia chamber. Sham animals were injected IP with saline at 0.1 mL daily for 7 days starting from P1.

Hematoxylin and eosin staining

Brain tissue was fixed on P14 in 4% paraformaldehyde for 24 h, processed, paraffin embedded, and sectioned sagittally at 6-μm-thickness. After deparaffinization, hematoxylin and eosin (H&E) staining was performed according to standard protocols. $N = 5$ mice per group.

Immunohistochemistry

Animals were injected at P14 with a lethal dose of xylazine/ketamine and perfused transcardially with saline, and then 4% paraformaldehyde (PFA). Whole brain tissue was fixed in 4% PFA for 24 h, processed, paraffin embedded and sectioned sagittally and coronally at 6 μm thickness. For immunofluorescence, after deparaffinization and antigen retrieval, sections were incubated for 2 h at room temperature (RT) in TBS + 1% Triton-X + 10% donkey serum. Next, sections were incubated for 24 h at 4°C with primary antibodies, followed by 2 h incubation at RT with the appropriate secondary antibody with DAPI. All images were captured on a Zeiss confocal microscope (Carl Zeiss, Thornwood, NY, USA). The following primary antibodies were used to detect the following markers: Anti-ChAT (Millipore {1:100} Billerica, MA, USA); GFAP (Abcam {1:500} Cambridge, MA, USA); Iba1 (Wako {1:400}, Richmond, VA, USA); CNPase (Abcam {1:200} Cambridge, MA, USA); Olig2 (Santa Cruz Biotechnology{1:50} Dallas, TX, USA) and secondary antibodies (Species specific Cy3 and FITC {1:125} Jackson Immuno-research, Westgroove, PA).For negative control, sections were incubated with TBS + 1% Triton-X + 10% donkey serum for 24 h (no primary antibody added) followed by 2 h incubation with the appropriate secondary antibody with DAPI.

Immunostaining analysis

Digital images were obtained using confocal software and then exported to Image J. Excitation and acquisition parameters were adjusted to fully eliminate pixel saturation and all images were collected under identical settings. Each section corresponds to 750 × 750 μm. The fluorescence intensity of each pixel was performed in 4 sections per mouse and 5 mice per group using image J.

Cell counting was performed using image J using plugins AnalyzeSkeleton (2D/3D). Skeletonized images are assessed for accuracy by creating an overlay of the skeleton and the original image. Cell counting was performed on 4 sections per animal (750 × 750 μm each) and 5 animals per group. Even though analysis was automated, all analysis was performed by one investigator for consistency who was blinded to the study group to eliminate bias.

Cytokines assay

Proinflammatory cytokines IL-1β, IL-6, and TNF-α ELISAs were done on cortical tissue on postnatal day 8 using Quantikine ELISA kits (R&D Systems) that were used according to the manufacturer's instructions. $N = 5$ animals/group.

Mouse HMGB1 chemiluminescence ELISA kit (Novus biologicals) was performed according to the manufacturer's instructions. $N = 5$ animals/group.

Western blot

Western blot was used to determine quantity of ChAT, phosphorylated p65 (marker of NF-κB activation) and P65 in cortical tissue homogenates on P8. After protein extraction, protein concentration was estimated using the Modified Lowry Protein Assay (Thermo Fisher Scientific, Rockford, IL, USA). Standard SDS-PAGE techniques were followed. After electrophoresis, proteins were transferred to a PVDF membrane using a Wet/Tank Blotting System (Bio-Rad, Hercules, CA, USA). Membranes were briefly washed, incubated with respective primary antibody in 5% BSA with PBST overnight. After washing, the membranes were incubated with HRP-conjugated secondary antibodies for 60 min, washed, processed using Amersham ECL detection systems (GE healthcare, Piscataway, NJ USA) and exposed to 8 × 10 Fuji x-ray Film. Density of ChAT band was presented as a ratio to Actin band density. Density of phosphoP65 was presented as a ratio to P65. The following primary antibodies were

used: ChAT antibody (Millipore {1:1000} Billerica, MA, USA); phospho p65 (Cell Signaling Technology {1:500}, Danvers, MA, USA); P65 (Cell Signaling Technology {1:500}, Danvers, MA, USA); and anti-Beta- Actin protein (as an internal control) (Cell Signaling Technology {1:1000}, Danvers, MA, USA). Horseradish Peroxidase (HRP)-Conjugated Goat Anti-Rabbit and goat anti-mouse IgG conjugate were used for detection of rabbit and mouse primary antibodies, respectively, (Bio-Rad {1:5,000}, Hercules, CA, USA). $N = 6$ mice/group.

Caspase 3 activity

Caspase 3 activity, as a marker for apoptosis, was measured on P8 with a Caspase-3 Colorimetric Assay (R&D Systems, Minneapolis, MN, USA). The assay was performed according to the manufacturer's instructions, as described previously (Gerstner et al., 2008). The fluorescence was measured at the excitation wavelength of 360 nm, emission wavelength of 460 nm using a multiplate fluorescence reader (Biotek Instruments). The protein concentration was measured with a Pierce kit. Acetylated-7-Amino-4-methylcoumarin (AC-AMC) was used for obtaining the standard curve. Enzyme activity was calculated as picomoles per minute per milligram of protein.

ROS assays

The DCFDA Intracellular ROS Assay (Abcam, Cambridge, MA, USA) was done per the manufacturer's instructions on P8. Clear tissue lysates are placed in a 96-well cell culture plate and then pre-incubated with DCFH-DA, a standard substrate. The sample lysates were then added to the DCFH-DA. After an incubation period of 30 min, the samples were read on a standard fluorescence plate reader at 480 nm excitation/538 nm emission. The ROS levels in the samples was determined by comparison with the predetermined DCF standard curve (Kobayashi et al., 2016).

H_2O_2 assay

A colorimetric hydrogen peroxide detection kit [Enzo Life Sciences (ADI-907-015)] was used for the assay according to the manufacturer's instructions. Standard concentrations of H_2O_2 were run along with the sample lysates. After incubation for 3 min, the absorbance was measured between 540 and 570. The samples' H_2O_2 content was determined by comparison with the predetermined H_2O_2 standard curve (Wentworth et al., 2001).

Acetylcholinesterase activity assay

Cortical brain tissue homogenates on P14 were prepared in a 20 mM Tris–HCl buffer (pH 7.3), containing 10 mM MgCl2, 50 mM NaCl, a protease inhibitor and zirconium beads using a bullet-blender homogenizer (Next Advance, Troy, NY, USA), according to the manufacturer's recommendations. Homogenates were centrifuged (24,500 g), pellets were resuspended in equal volumes of 0.1 M NaHPO4 buffer (pH 10), then centrifuged again at (24,500 g), using the resultant supernatants (containing membrane bound AChE). AChE activity assay based on the widely used Ellman's procedure (Guarini et al., 2004) and its modification was followed (Lee et al., 2010). A butyrylcholinesterase inhibitor was added to the reaction mix. The reaction was initiated by adding acetylthiocholine substrate, a thiolester. The thiocholine amount formed reflects AChE activity. The reaction mix color was read at 412 nm. Calculations using molar absorptivity (Ɛ) for thionitrobenzoate (TNB) were done at 412 nm. The results are expressed as mM of thiocholine released per minute at 25°C per 1 mL of lysate per 1 mg of protein. For positive control, dilute AChE was added instead of the supernatant and the same procedure was followed.

Neurobehavioral testing and long-term outcome

Rotarod device (Columbus Instruments, Columbus, OH, USA) was performed at P60 to assess mice motor function, coordination and balance. Each session consisted of the average of three trials on the elevated accelerating rotarod beginning at 5 RPM, measuring the time the mouse was able to remain on the rod. At P60, mice were tested in an open field analysis (San Diego Instruments, San Diego, CA, USA). Mice had one 30 min training session before the beginning of testing, to adapt to the testing chamber. Open field data was digitally recorded for 30 min and then analyzed by Noldus Ethovision tracking software (Hartman et al., 2009). Beam breaks were recorded in the *x*, *y*, and *z* planes and averaged across groups. Novel object recognition test was performed on P60 to evaluate learning and memory. Mice were adapted to empty box once a day for 3 days, then adapted to a box containing 2 identical objects once a day for 3 days. One of the objects in the box was replaced by a new one for which they were adapted also once a day for 3 days. Then testing began. When mice sniffed or touched the objects, but not climbed over the objects, it was considered an effective exploration. The exploration time was recorded by two blinded observers to treatment/exposure allocation. The "recognition index" was out according to a formula: (exploring novel object time- exploring familiar object time)/(total exploration time for novel and familiar objects) (Mei et al., 2020). $N = 25$ mice/group.

Statistical analysis

For all statistical tests, Graph Pad Prism 8 software (La Jolla) was used. Statistical analysis of mean differences between groups was performed by using student *t*-test or one-way ANOVA, followed by a Bonferroni post-hoc analysis. All *p* values and *n* values are indicated in figure legends.

Two hundred twenty total pups were used for all experiments. Male/female ratio is 6:5. No sex difference was found in the studies.

Results

Oxygen therapy is a double edge sword for preterm infants. It is essential for the treatment of their respiratory distress, at the same time causing bronchopulmonary dysplasia, cognitive dysfunction and free radical injury to all organs (Mohamed et al., 2020). We hypothesized that galantamine would ameliorate this hyperoxic brain injury, while improving cognition and memory in a neonate mouse model of hyperoxia (Zaghloul et al., 2012). There was no mortality in any of the mice groups.

To better elucidate the central cholinergic actions of galantamine, we show a simplified schematic diagram of the cholinergic system of the brain (Figure 1A). A sagittal section of the brain with cholinergic

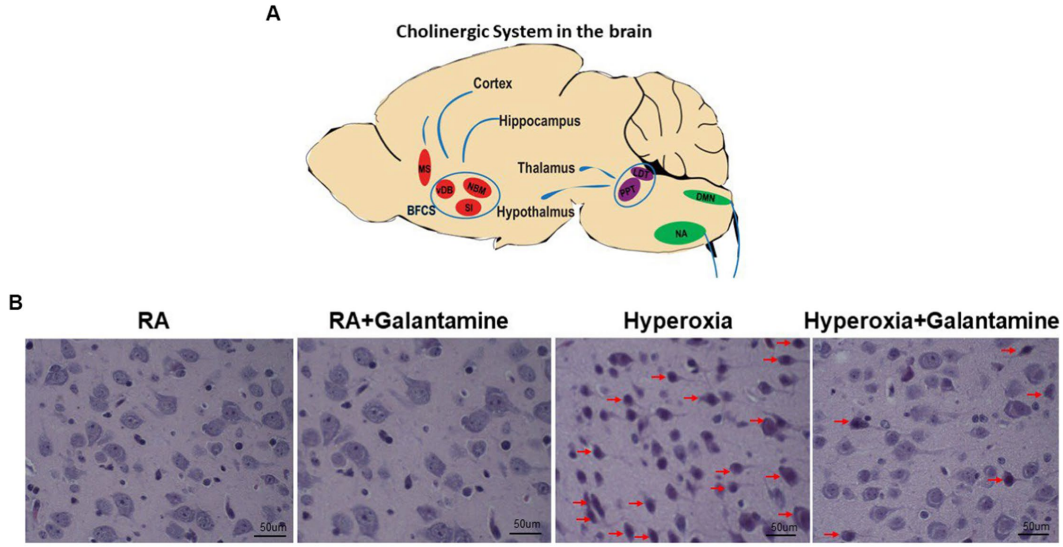


FIGURE 1
Galantamine rescues cholinergic neurons from hyperoxia induced neurodegeneration. **(A)** Simplified schematic diagram of the Cholinergic system of the brain. Sagittal view from anterior (1) to posterior (6). Medial septum (MS). Basal forebrain cholinergic system (BFCS) which consist of the vertical limbs of the diagonal band of Broca (vDB), substantia innominate (SI) and nucleus basalis of Meynert (NBM), projecting to the cortex and hippocampus. Brain stem cholinergic nuclei: Pedunclopontine tegmental (PPT) nucleus and Laterodorsal tegmental (LDT) nucleus. Both PPT and LDT innervate thalamus, hypothalamus and hindbrain. Nucleus amibiguus (NA) Dorsal motor nucleus of the vagus (DMN). NA and DMN provide cholinergic projection with the vagus nerve to the periphery (heart, lung, liver, and other organs supplied by the vagus nerve). **(B)** H&E stain on P14 of the basal forebrain cholinergic system showing cholinergic nuclei of room air (RA), hyperoxia and hyperoxia+ galantamine groups. Red arrows indicate degenerating cholinergic nuclei. Hyperoxia causes extensive neurodegeneration of cholinergic nuclei which is rescued by galantamine administration. Magnification 40x. Scaler bar = 50 μm. *N* = 5 animals/group & 4 sections/animal.

nuclei from anterior to posterior is provided in Figure 1A. Medial septum (MS). Basal forebrain cholinergic system (BFCS) which consist of the vertical limbs of the diagonal band of Broca (vDB), substantia innominate (SI) and nucleus basalis of Meynert (NBM), projecting to the cortex and hippocampus. Brainstem cholinergic nuclei: Pedunculopontine tegmental (PPT) nucleus and Laterodorsal tegmental (LDT) nucleus. Both PPT and LDT innervate thalamus, hypothalamus and hindbrain. Nucleus amibiguus (NA) and Dorsal motor nucleus of the vagus (DMN). NA and DMN provide cholinergic projection with the vagus nerve to the periphery (heart, lung, liver and other organs supplied by the vagus nerve).

Galantamine rescues cholinergic neurons from hyperoxia induced neurodegeneration

Hyperoxia causes cortical, hippocampal and cerebellar neuronal damage and degeneration in the form of apoptosis and necrosis (Gerstner et al., 2008). H&E staining of cholinergic cells showed neuronal degeneration of the Basal forebrain cholinergic system in hyperoxic non-treated group (red arrows in Figure 1B). Galantamine treatment rescues cholinergic neurons from hyperoxia induced neuronal degeneration as shown in Figure 1B. There is no difference between RA+ saline and RA+ galantamine group.

H&E staining of cholinergic cells showed neuronal degeneration of the Lateral Dorsal Tegmental nuclei (LDT, upper panel) and Nucleus Ambiguus (NA, lower panel) in hyperoxic non-treated group (red arrows in Supplementary Figure S1). Galantamine treatment rescues cholinergic neurons from hyperoxia induced neuronal degeneration (Supplementary Figure S1).

Galantamine rescues cholinergic nuclei from hyperoxia induced neuronal loss

Hyperoxia causes cholinergic neuronal damage and degeneration which leads to apoptosis and necrosis and eventually neuronal loss (Reich et al., 2016). Examining the cholinergic system by assessing cholinergic neuronal cell number was done by immunofluorescent staining for Choline acetyltransferase (ChAT), the enzyme that is responsible for the synthesis of acetyl choline (Ach), the main neurotransmitter for the parasympathetic system. We show that hyperoxia caused a significant loss of ChAT positive cells in the BFCS, while treatment with Galantamine, prevented loss of ChAT positive neurons after hyperoxia exposure (Figures 2A,B). Hyperoxia also caused a significant loss of ChAT positive cells in LDT (Figures 2C,D), and NA (Figures 2E,F), while Galantamine treatment in this hyperoxic setting rescued ChAT positive neuron in the BFCS, LDT and NA to the RA numbers. There was no difference between the 2 RA groups in all three cholinergic nuclei (Figure 2). The loss of ChAT positive neurons leads to a decrease in Ach and thus parasympathetic innervation to the areas innervated by the BFCS, LDT and NA.

Galantamine ameliorates hyperoxia induced reductions in cholinergic brain activity

Hyperoxia significantly decreases ChAT protein expression in the BFCS as shown by western blot study, while ChAT protein levels were preserved in hyperoxia group treated with Galantamine, with ChAT protein levels equivalent to RA group (Figure 3A). ChAT protein

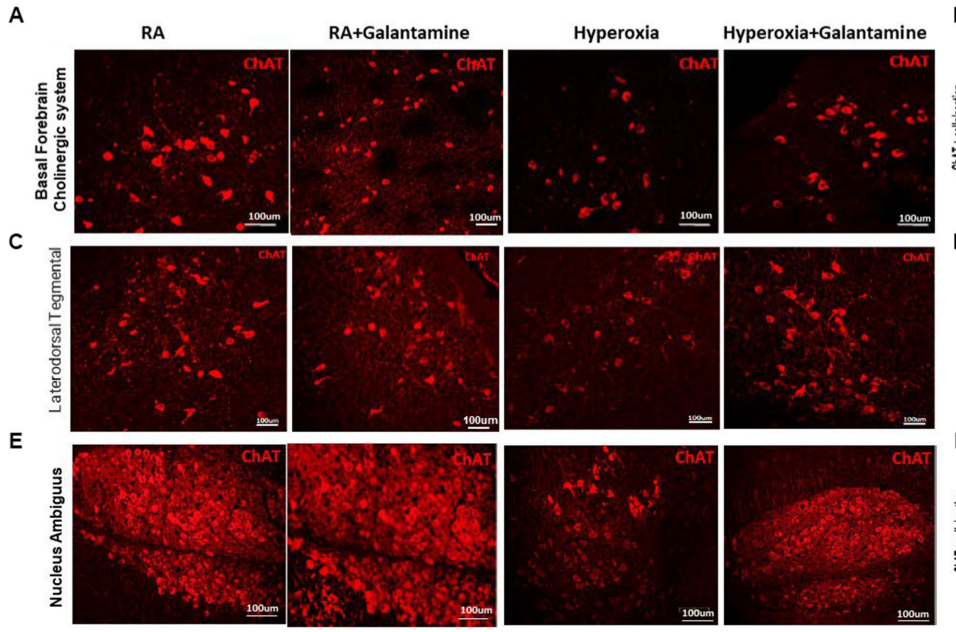


FIGURE 2
Galantamine rescues cholinergic nuclei from hyperoxia induced neuronal loss. **(A)** Immunohistochemistry of choline acetyltransferase containing cells (ChAT) of the Basal forebrain cholinergic system (BFCS) at P14 in all study groups. Scale bar = 100 μm. **(B)** Quantification of ChAT positive cells per section in BFCS. *N* = 5 animals/group & 4 sections/animal. Scatter dot plot showing mean ± SE. *$p \leq 0.05$, ****$p \leq 0.0001$. **(C)** Immunohistochemistry of choline acetyltransferase containing cells (ChAT) of Laterodorsotegmental (LDT) nucleus at P14 in all study groups. Scale bar = 100 μm. **(D)** Quantification of ChAT positive cells per section in LDT nucleus. *N* = 5 animals/group & 4 sections/animal. Scatter dot plot showing mean ± SE., ****$p \leq 0.0001$. **(E)** Immunohistochemistry of choline acetyltransferase containing cells (ChAT) of Nucleus ambiguous nucleus (NA) at P14 in all study groups. Scale bar = 100 μm. **(F)** Quantification of ChAT positive cells per section in NA nucleus. *N* = 5 animals/group & 4 sections/animal. Scatter dot plot showing mean ± SE., ****$p \leq 0.0001$.

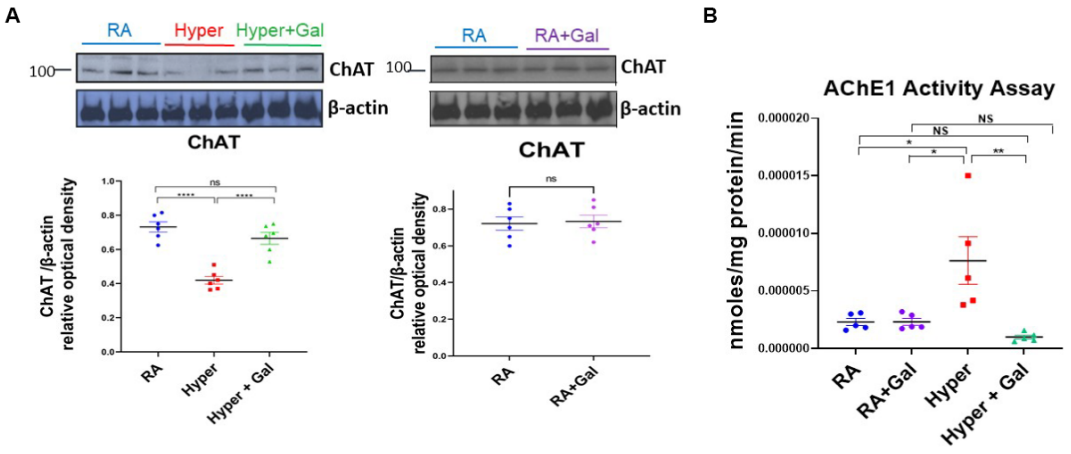


FIGURE 3
Galantamine ameliorates hyperoxia induced reductions in cholinergic brain activity. **(A)** ChAT western blot of cortical protein lysate at P14 in RA, Hyperoxia and Hyperoxia +Galantamine groups, represented as a ratio to β -actin protein. Scatter dot plot densitometry quantification analysis represented as ChAT/β -actin relative optical density showing mean ± SE., ****$p \leq 0.0001$. ChAT western blot of cortical protein lysate in RA + Saline group as compared to RA + Galantamine group represented as ChAT/β - actin relative optical density showing mean ± SE., P is ns. *N* = 6 animals/group. **(B)** Acetylcholinesterase activity assay of cortex presented as nmol/mg protein/min at P14 in all study groups. *N* = 5 animals/group Scatter dot plot showing mean ± SE., *$p \leq 0.05$, **$p < 0.01$.

expression levels (Figure 3A) coincides with ChAT neuronal staining in the BFCS (Figures 2A,B). Acetylcholine esterase breaks down acetylcholine. As acetylcholine esterase activity increases, ChAT protein expression decreases. To explore if that is in fact the reason for the decreased in ChAT protein expression, we analyzed BFCS acetylcholine esterase activity. In hyperoxia group, acetylcholine esterase activity was significantly increased in comparison to both RA groups and Galantamine hyperoxia treated group. Galantamine treated hyperoxia group had similar acetylcholine esterase levels to both RA groups (Figure 3B).

Galantamine has an anti-inflammatory effect on glia in hyperoxic environment

Microgliosis and microglial activation potentiates neuroinflammation by releasing pro- inflammatory cytokines (Zaghloul and Ahmed, 2017). Hyperoxia resulted in microgliosis (indicated by Iba1 staining) especially in the white matter where microglia are more abundant. Galantamine treated hyperoxia group shows a significant reduction in microgliosis in the periventricular white matter as compared to the hyperoxia group. No difference was found in microglial number between Galantamine treated hyperoxia group and RA groups (Figures 4A,B). There is also no difference in microglial numbers between RA and RA + Galantamine groups.

Astrogliosis causes blood brain barrier disruption leading to formation of the glial scar (Zhou et al., 2020). Hyperoxia caused significant astrogliosis shown in the CA1 area of the hippocampus and dentate gyrus as indicated by the increase in GFAP staining in the hyperoxia group hippocampal region compared to the RA groups. Galantamine treatment significantly reduced astrogliosis induced by hyperoxia in comparison to non-treated hyperoxia group (Figures 4C,D). Galantamine administration to RA group has no effect on astrocytes.

Myelination of the hippocampus leads to improvements of both learning and memory. Galantamine treatment to RA group did not alter pre-oligodendrocyte or oligodendrocyte numbers. We show a significant reduction in pre-oligodendrocytes in the CA1 area of the hippocampus and dentate gyrus indicated by CNPase staining in the hyperoxia group compared to the RA group. Pre-oligodendrocytes were preserved in the hyperoxia group treated with Galantamine (Figures 4E,F). Oligodendrocytes indicated by Olig −2 staining in the periventricular white matter area were also significantly decreased in the hyperoxia group as compared to both RA groups. Galantamine reduced oligodendrocytes loss caused by hyperoxia, thus ameliorating white matter loss (Figures 4G,H).

Galantamine attenuates neuro-inflammation, apoptosis, and oxidative stress in hyperoxic environment

Proinflammatory cytokines namely IL-1β, IL-6, TNFα, were significantly increased in the cortex of hyperoxic pups as compared to the RA groups. Galantamine treated hyperoxia group had a significant reduction in the levels of proinflammatory cytokines as compared to hyperoxia group (Figure 5A). Brain high mobility group box 1 (HMBG1) is one of the Damage Associated Molecular Patterns (DAMP) secreted by microglia and is an initiator and amplifier of neuroinflammation (Gou et al., 2020). HMBG1 was significantly increased in hyperoxia group as compared to RA group. Galantamine treated hyperoxia group had a significant decline in HMBG1 as

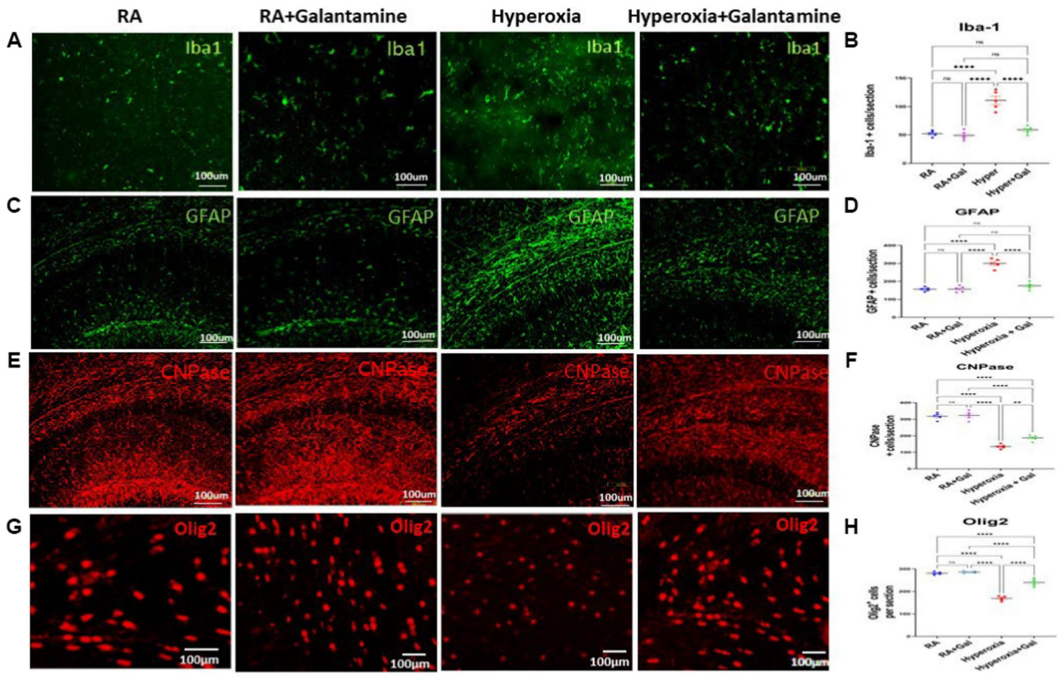


FIGURE 4
Galantamine has an anti-inflammatory effect on glia in hyperoxic environment **(A)** Immunohistochemistry of microglia (Iba1 in green) in the periventricular white matter area on P14 in all study groups. Scale bar = 100 µm. **(B)** Quantification of Iba-1 positive cells per section. *N* = 5 animals/group & 4 sections/animal. Scatter dot plot showing mean ± SE. ****$p \leq 0.0001$. **(C)** Immunohistochemistry of astrocytes (GFAP in green) in the CA1 area of the hippocampus and dentate gyrus on P14 in all study groups. Scale bar = 100 µm. **(D)** Quantification of GFAP intensity as fold change where RA = 1. *N* = 5 animals/group & 4 sections/animal. Scatter dot plot showing mean ± SE. ****$p \leq 0.0001$. **(E)** Immunohistochemistry of Pre-oligodendrocytes (CNPase in red) in the CA1 area of the hippocampus and dentate gyrus on P14 in all study groups. Scale bar = 100 µm. **(F)** Quantification of CNPase intensity as fold change where RA = 1. *N* = 5 animals/group & 4 sections/animal. Scatter dot plot showing mean ± SE. ****$p \leq 0.0001$. **(G)** Immunohistochemistry of oligodendrocytes (Olig-2 in red) in the periventricular white matter area on P14 in all study groups. Scale bar = 100 µm. **(H)** Quantification of Olig-2 positive cells per section. *N* = 5 animals/group & 4 sections/animal. Scatter dot plot showing mean ± SE. **$p < 0.001$, ****$p \leq 0.0001$.

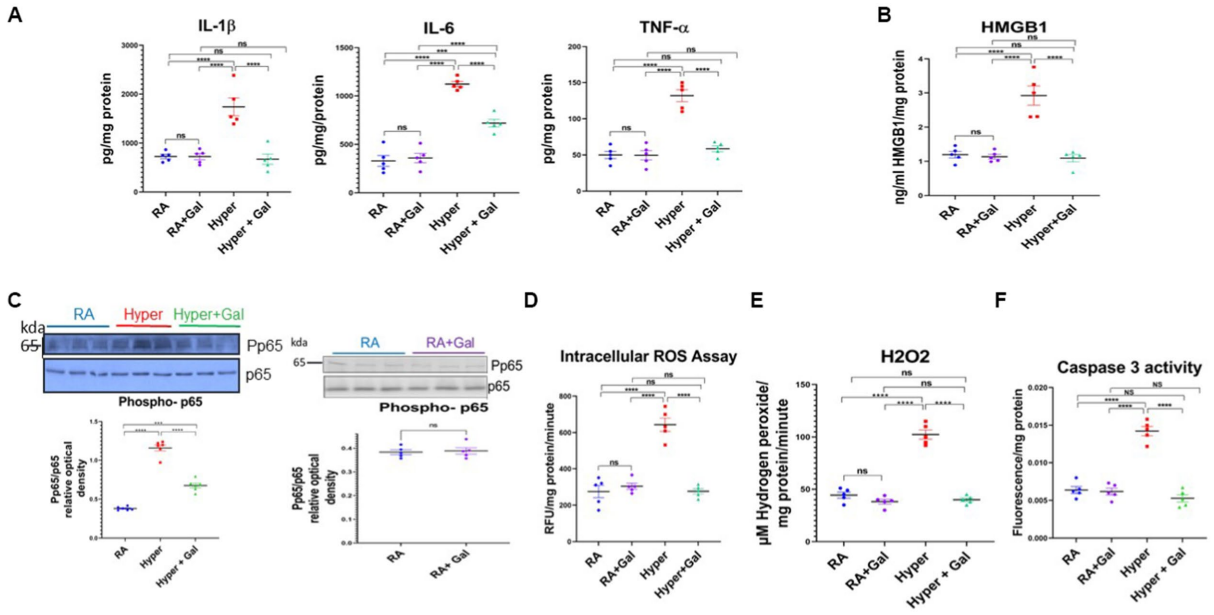


FIGURE 5
Galantamine attenuates neuro-inflammation, apoptosis and oxidative stress in hyperoxic environment. **(A)** Brain pro-inflammatory cytokine levels IL1β, IL-6, TNF α measured by ELISA from cortical brain lysates on P8 in all study groups. *N* = 5 animals/group. Scatter dot plot showing mean ± SE. ****$p \leq 0.0001$. **(B)** Brain high mobility group box 1 (HMGB1) assay by ELISA from cortical brain lysates on P8 in all study groups. *N* = 5 animals/group. Scatter dot plot showing mean ± SE. ***$p < 0.001$, ****$p \leq 0.0001$. **(C)** Brain Phosphorylated P65 western blot represented as a ratio to p65 protein at P8 of cortical brain lysates in RA, Hyperoxia, Hyperoxia + Galantamine. Brain Phosphorylated P65 western blot represented as a ratio to p65 protein at P8 of cortical brain lysates in RA + Saline and RA + Galantamine *N* = 6 animals/group. Scatter dot plot showing mean±SE. P ns, ***$p < 0.001$, ****$p \leq 0.0001$. **(D)** DCFDA intracellular ROS assay, measuring superoxide and hydroperoxyl expressed as relative florescence units (RFU) per mg protein per minute in neonate mouse cortex on P8 in all study groups. *N* = 5 animals/group. Scatter dot plot showing mean ± SE. ****$p \leq 0.0001$. **(E)** Hydrogen peroxide ROS assay measuring µM hydrogen peroxide per mg protein per minute in neonate mouse cortex on P8 in all study groups. *N* = 5 animals/group. Scatter dot plot showing mean ± SE. ****$p \leq 0.0001$. **(F)** Activated Caspase 3 activity by ELISA measuring apoptosis in neonate mouse brain cortex tissue on P8 of cortical brain lysates in all study groups. *N* = 5 animals/group. Scatter dot plot showing mean ± SE. ****$p \leq 0.0001$.

compared to hyperoxia group (Figure 5B). These finding indicate that Galantamine attenuates neuro-inflammation. Hyperoxia exposure caused a significant increase of phosphorylated p65/p65, indicating NF-KB activation when compared to RA groups. Galantamine treated hyperoxia group had a significantly lower phosphorylated p65/p65 when compared to hyperoxia group (Figure 5C). There is no difference in cytokine levels, HMGB1 or NF-KB activation between the two RA groups. These findings highlight the anti-inflammatory role of Galantamine. Compared to the two RA groups, hyperoxia caused a significant increase in ROS levels assessed by both Oxiselect intracellular ROS assay, (measures intracellular superoxide and hydroperoxyl radical), and H_2O_2 assay (measures hydrogen peroxide). Galantamine treated hyperoxia group had significantly lower ROS levels than hyperoxic non-treated group (Figure 5D). These data support the anti-oxidative role of Galantamine.

Increase in ROS production overwhelms the cellular antioxidant capacity and results in damage to the DNA, proteins and lipids leading to apoptosis and necrosis (Thannickal and Fanburg, 2000). Thus, caspase 3 activity was measured to assess apoptosis in all studied groups. Caspase 3 activity was significantly increased in the hyperoxia group as compared to the RA groups. Galantamine treated hyperoxia group had a significantly lower caspase 3 activity when compared to hyperoxia group, with levels similar to RA group (Figure 5F). Galantamine treatment to the RA group had no difference in ROS levels or apoptosis, in comparison to RA saline group. These findings suggest anti-apoptotic activity of galantamine.

To evaluate late stages of neuronal apoptosis and neuronal necrosis, we performed Tunel staining. We examined Tunel colocalization with ChAT positive cholinergic nuclei in the BFCS as an indication of cholinergic neuronal necrosis. Tunel staining was significantly increased in the hyperoxia BFCS ChAT positive nuclei as compared to the RA groups. Galantamine treated hyperoxia group had a significantly lower Tunel staining when compared to hyperoxia group, thus providing protection against cholinergic neuronal necrosis (Supplementary Figure S2).

Galantamine improves long term neurodevelopmental outcomes in hyperoxia

To investigate if the improvement in histopathological findings, cholinergic activity and oligodendrocyte numbers, along with reduced neuro-inflammation and oxidative stress, translate to improvement in neurodevelopmental outcomes, neurobehavioral analysis was performed at P60. On rotarod, hyperoxia group had impaired coordination as they had a more tendency to fall from the rotarod, while Galantamine treated hyperoxia group had less tendency to fall, thus improved co-ordination than the hyperoxic group (Figure 6A). Open field analysis shows a significantly reduced number of beam breaks, indicating worsening locomotion in hyperoxia versus hyperoxia Galantamine mice group (Figure 6B). Preference index for the novel

object in the novel object recognition test was significantly increased in Galantamine treated hyperoxia group than non-treated hyperoxia group (Figure 6C). Mice with better recognition memory spend more time exploring a novel object than a familiar one. Galantamine administration to RA showed no significant difference to RA group in all the above neurobehavioral outcomes measured. This data indicated improved memory function especially recognition memory in Galantamine treatment hyperoxia group versus non treated one.

Since we showed that Galantamine improves memory function, therefore we aimed to measure hippocampal volume at P60, to correlate with the novel object recognition test. Hyperoxia significantly decreases hippocampal volumes measured on T2 weighted images. Galantamine treated hyperoxia group had larger hippocampal volumes as compared to hyperoxia alone (Supplementary Figure S3). RA and RA + Galantamine groups had no difference in hippocampal volumes. Hyperoxia can cause severe and permanent retinal degeneration. We show that in hyperoxia, the hyaloid artery was hyperplastic and extended onto the retina near the optic nerve head and formed a thick distinct capsule on the posterior lens surface. Outer retinal degeneration with elevated and disrupted retinal layers and neovascularization was evident in hyperoxic pups. In Galantamine treated pups that undergone hyperoxia, the hyaloid artery was less hyperplastic, also formed a capsule on the posterior lens surface, and the outer retinal cell disruption and neovascularization was less severe (Supplementary Figure S4).

Discussion

Our study provides strong evidence that supports the protective role of galantamine in hyperoxic brain injury in neonate mice. Galantamine is a reversible and competitive acetyl choline esterase inhibitor and a positive allosteric modulator of nicotinic acetylcholine receptors. Galantamine inhibits the AChE from dissociating acetyl choline, therefore increasing the neurotransmitter acetyl choline level and duration of action (Colović et al., 2013). In hyperoxic mice, we showed a significant increase of AChE activity as compared to RA and galantamine treated hyperoxia groups (Figure 3B). This finding was accompanied by a significant reduction of ChAT expression as shown by Western Blot assay and immunostaining (Figures 2, 3A).

Galantamine potent anti-inflammatory properties is mainly attributed to its ability to increase acetylcholine level and activity, a known anti-inflammatory neurotransmitter, through the inhibition of AChE. Another anti-inflammatory mechanism of galantamine is inhibition of microgliosis, astrogliosis, pro-inflammatory cytokines and NF-κB activation induced by hyperoxia.

Premature infants are at high risk for infection/inflammation as they are often born to mothers with chorioamnionitis. They have very low innate and acquired immunity as compared to full term infants (Villamor-Martinez et al., 2020).

We showed an overall reduction in inflammatory response related to hyperoxia stress in the galantamine treated group through significant reduction of different inflammatory cytokines including IL-1β, IL-6, TNFα, pNF-κB, and HMGB1 compared to non-treated hyperoxia group (Figures 5A–C). Our findings support the anti-inflammatory properties of galantamine, which were also shown previously, in neonatal hypoxic ischemic rats (Furukawa et al., 2014). Galantamine, therefore restores the normal milieu for brain development and brain cell proliferation and maturation.

The immature brain is extremely vulnerable to ROS (produced by hyperoxia) due to high oxygen consumption rate, high concentrations of unsaturated fatty acids, low levels and activity of antioxidants, and large amount of susceptible immature cells (Collins et al., 2001). Galantamine acts as an antioxidant by decreasing intracellular ROS levels through decreasing ROS overproduction and scavenging of already produced ROS (Figure 5D).

Galantamine increases acetylcholine levels and phosphorylates serine–threonine protein kinase. Through stimulation of phosphoinositide 3-kinase and increased expression of protective protein Bcl-2, galantamine decreases overproduction of ROS (Mei et al., 2020). Galantamine scavenges reactive oxygen species by

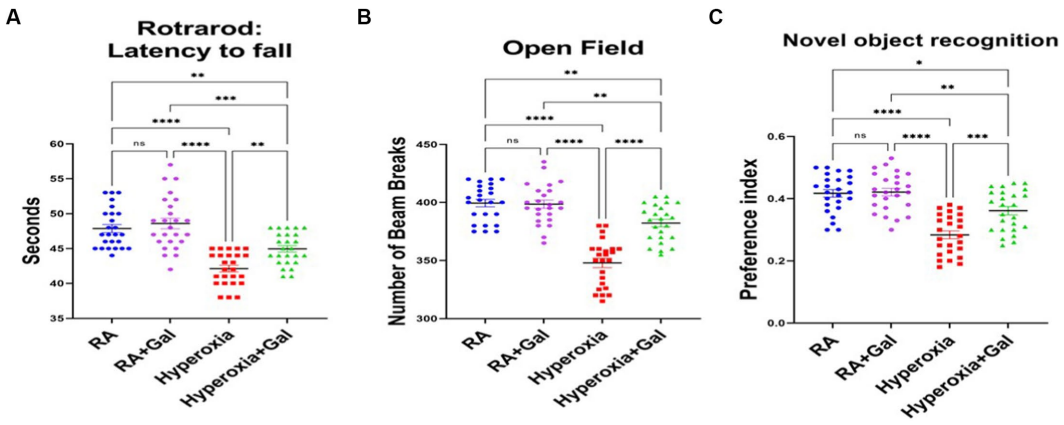


FIGURE 6
Galantamine improves long term neurodevelopmental outcomes in hyperoxia. **(A)** Testing of latency to fall in seconds by rotarod for coordination assessment at P60. in all study groups. *N* = 25 animals/group. Scatter dot plot showing mean ± SE. ***$p \leq 0.001$, ****$p \leq 0.0001$. **(B)** Number of beam breaks in the open field test as an assessment of motor function at P60 in all study groups. *N* = 25 animals/group. Scatter dot plot showing mean ± SE. **$p \leq 0.01$, ****$p \leq 0.0001$. **(C)** Showing preference index of novel object recognition as a measure of memory at P60 in all study groups. *N* = 25 animals/group. Scatter dot plot showing mean ± SE. **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

inhibiting the activation of P2X7 receptors, stabilizing mitochondrial membrane potential and preventing membrane fluidity disturbance (Tsvetkova et al., 2013). Oxygen therapy cannot be avoided in neonatal intensive care. Therefore, when oxygen supplementation is required, in addition to developing appropriate monitoring systems, protective and/or regenerative strategies are highly warranted.

Neuroinflammation and excessive ROS causes pre-oligodendrocyte injury and death leading to eventual white matter loss (Zaghloul et al., 2017) Pre-oligodendrocytes are the most sensitive and vulnerable to oxidative stress and neuroinflammation. As anticipated, reduction of the free radicals and attenuation of the inflammatory response through galantamine administration plays significant role in preserving oligodendrocytes and myelination.

Oxidative stress induces direct brain cell injury in both grey and white matter. Hyperoxia induces upregulation of inducible nitric oxide synthetase (iNOS) mRNA which is mainly synthesized by microglia (Hoehn et al., 2003). Free radicals and increased production of nitric oxide (NO), both induce cell death and DNA fragmentation in brain tissue (Platt and Riedel, 2011). In our hyperoxic neonate group, there was significant apoptosis compared to the RA groups (Figure 5F). Our results show that administration of galantamine during hyperoxia led to a significant reduction of ROS levels (Figure 5D) and inhibition of apoptosis as shown by reduction of caspase 3 activity (Figure 5E) as well as a protection of cholinergic neurons from hyperoxia induced neurodegeneration (Figure 1B). Further studies investigating the effect of hyperoxia and galantamine administration on neuronal and glial cell proliferation and maturation are needed.

Brain cholinergic system plays a significant role during neonatal brain development by modulating neurogenesis, gliogenesis, synaptic plasticity, attention, learning, memory, REM sleep (Platt and Riedel, 2011) and the control of movements. Disruption of the cholinergic system during brain development, leads to adverse effects on brain development in neonates especially premature infants (Abreu-Villaça et al., 2011). Hyperoxia leads to neuronal and glial cell death, resulting in white and grey matter brain injury observed in preterm infants. During the critical phase of brain maturation, hyperoxia can alter developmental processes, disrupting neural plasticity and myelination (Reich et al., 2016). Our study had similar findings to those seen in preterm infants, showing significant reduction of myelination (Olig-2 staining) with hyperoxia and myelin preservation when galantamine is administered to hyperoxia group.

In our study, galantamine administration during hyperoxia exposure leads to preservation of ChAT levels. This rescue of ChAT+ cells in the BFCS (Figure 2A) which gives cholinergic innervation to neocortex and hippocampus (Figure 1A) leads to improvement of learning and memory as shown by increased preference index for the novel object in the novel object recognition test (Figure 6C). The rescue of ChAT in the LDT which supplies the thalamus, hypothalamus and hindbrain improves motor function (Figure 6B) and REM sleep. All cholinergic nuclei contribute a significant role in reducing inflammation (Figures 5A–C), improving myelination (Figures 4G,H) and memory (Figure 6C). Hippocampal volume is significantly preserved and protected from the damaging effects of hyperoxia through increasing the BFCS's cholinergic innervation to the hippocampus by Galantamine administration (Supplementary Figure S3). This, along with decreased neuro-inflammation, neuronal apoptosis and ROS, led to improved hippocampal volume and memory function. Restoring cholinergic system integrity, as well as activity after galantamine administration to the hyperoxic pups, has a positive effect as shown in our long-term neuro- developmental studies (Figure 6). Similar role was shown after daily Galantamine administration in experimental traumatic brain injury, which showed significant improvements in cognitive deficits and histological recovery (Zhao et al., 2018).

Galantamine potentiates "cholinergic anti-inflammatory pathway." This pathway inhibits cytokine release by neural signals, transmitted via the vagus nerve, through a mechanism that requires the alpha7 subunit-containing nicotinic acetylcholine receptor (α7nAChR). This reflex suppresses NF-κB activation and pro-inflammatory cytokine production (Tracey, 2002; de Jonge and Ulloa, 2007; Pavlov and Tracey, 2015). Therefore, in addition to inhibiting neuro inflammation, Galantamine can inhibit systemic inflammation. Since hyperoxia has deleterious effects on all organs in premature infants especially lung (Buczynski et al., 2013) and eyes, galantamine can ameliorate hyperoxia induced injury to those organs.

The reduced performance in open field exploration, novel object recognition in hyperoxic mice is likely a combination of damage to the cholinergic nuclei in addition to retinopathy and retinal degeneration. Galantamine ameliorated this retinopathy, likely contributing to the improved neurobehavioral outcomes. Further detailed studies need to be carried out to provide such evidence.

A limitation of this study is that our neonatal hyperoxia mouse model uses 95% oxygen for 7 days, while premature infants in the neonatal intensive care unit are sometimes exposed to much lower oxygen and still develop brain injury and poor neurodevelopmental outcome. We used this exaggerated hyperoxia condition, as proof of concept. Studies using 80%, even 65% oxygen are showing comparable results.

The results from our study are important as a preclinical model for controlled clinical trials in infants born very preterm. Their brains, even if ventilated with very high concentrations of oxygen, is never exposed to the very PaO_2 of the experiments reported here, as FiO_2 is titrated according to preductal arterial oxygen saturations using target ranges that are lower than normal values in healthy adults. Several large randomized controlled trials (SUPPORT, and BOOST-II) comparing higher versus lower target ranges of arterial oxygen saturation failed to find differences in neurodevelopmental outcome. However, A 3-4fold rise of PaO_2 occurs in virtually all mammals after birth when the source of oxygen switches from the placenta to the lungs, and preterm infants are poorly equipped to deal with sudden and profound surges of oxygen.

In conclusion, premature infants are at high risk for hyperoxia leading to systemic inflammation and neuroinflammation. Activation of 'the cholinergic anti-inflammatory pathway' by galantamine administration can improve short- and long-term outcomes in this susceptible population. In addition, galantamine has potent anti-inflammatory and antioxidant effects in hyperoxia-induced neonatal brain injury. It decreases oligodendrocyte loss thus preserving myelination. Increasing cholinergic activity by galantamine improves neuronal survival and increases ChAT expression. Long term neurobehavioral studies show that when galantamine administration in hyperoxia improves locomotion, coordination, learning and

memory. Our data suggest a prospective, innovative approach where galantamine could be clinically studied and applied as a new therapeutic agent in neonates with or at high risk for developing hyperoxia brain injury.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of the Feinstein Institute for Medical Research and the University of Arizona.

Author contributions

NZ, NC, DK, KA, H-LL, and MA conceived the project and designed the experiments. NZ and NC wrote the manuscript. NZ, NC, KA, and MA performed all *in vivo* experiments. NZ, NC, and H-LL performed the immunostaining and all statistical analyses. KA performed the cytokine protein expression. NZ performed all the neurobehavioral testing. NZ, DK, H-LL, and MA revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.890015/full#supplementary-material

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Glossary

Ach	Acetylcholine
AchE	Acetylcholine esterase enzyme
BFCS	Basal forebrain cholinergic system
CNPase	2',3'-Cyclic-nucleotide 3'-phosphodiesterase
ChAT	Choline acetyl transferase
CP	Cerebral palsy
DAMP	Damage Associated Molecular Patterns
DMN	Dorsal motor nucleus of the Vagus
LDT	Laterodorsal tegmental
Gal	Galantamine
GFAP	Glial fibrillary acidic protein
H_2O_2	Hydrogen peroxide
HMBG1	Brain high mobility group box 1
Iba1	Ionized calcium-binding adapter molecule 1
iNOS	Inducible nitric oxide synthetase
NA	Nucleus ambiguus
PPT	Pedunculopontin tegmentaltegmental
RA	Room air
ROS	Reactive oxygen species
WMI	White matter injury

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Therapeutic management of severe spinal cord decompression sickness in a hyperbaric center

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Introduction: Spinal cord decompression sickness (scDCS) unfortunately has a high rate of long-term sequelae. The purpose of this study was to determine the best therapeutic management in a hyperbaric center and, in particular, the influence of hyperbaric treatment performed according to tables at 4 atm (Comex 30) or 2.8 atm abs (USNT5 or T6 equivalent).

Methods: This was a retrospective study that included scDCS with objective sensory or motor deficit affecting the limbs and/or sphincter impairment seen at a single hyperbaric center from 2010 to 2020. Information on dive, time to recompression, and in-hospital management (hyperbaric and medical treatments such as lidocaine) were analyzed as predictor variables, as well as initial clinical severity and clinical deterioration in the first 24 h after initial recompression. The primary endpoint was the presence or absence of sequelae at discharge as assessed by the modified Japanese Orthopaedic Association score.

Results: 102 divers (52 ± 16 years, 20 female) were included. In multivariate analysis, high initial clinical severity, deterioration in the first 24 h, and recompression tables at 4 atm versus 2.8 atm abs for both initial and additional recompression were associated with incomplete neurological recovery. Analysis of covariance comparing the effect of initial tables at 2.8 versus 4 atm abs as a function of initial clinical severity showed a significantly lower level of sequelae with tables at 2.8 atm. In studying correlations between exposure times to maximum or cumulative O2 dose and the degree of sequelae, the optimal initial treatment appears to be a balance between administration of a high partial pressure of O2 (2.8 atm) and a limited exposure duration that does not result in pulmonary oxygen toxicity. Further analysis suggests that additional tables in the first 24–48 h at 2.8 atm abs with a Heliox mixture may be beneficial, while the use of lidocaine does not appear to be relevant.

Conclusion: Our study shows that the risk of sequelae is related not only to initial severity but also to clinical deterioration in the first 24 h, suggesting the activation of biological cascades that can be mitigated by well-adapted initial and complementary hyperbaric treatment.

KEYWORDS

diving, decompression sickness, bubbles, spinal cord, neurological sequelae, hyperbaric oxygen therapy, helium, lidocaine

Introduction

Among the serious pathologies specific to SCUBA diving, that require hospitalization, decompression sickness (DCS) is the most frequent and presents a significant morbidity with sometimes poor neurological prognosis (1–3). DCS encountered by divers is caused by the formation of bubbles from inert gas initially dissolved in the tissue during hyperbaric exposure. DCS injuries are rare with a prevalence of 1 to 3 per 10,000 dives (3). However, they often occur despite no violation of decompression procedures which limit their prevention. Spinal cord DCS, which presents with neurological symptoms, is the most frequent, accounting for about 50% of clinical DCS forms, and about 30% of long-term neurological sequelae (4, 5). The pathophysiological mechanisms are not fully understood, but result in localized spinal cord ischemia initially triggered by the formation of tissue or vascular bubbles (6).

The hospital management recommended by the most recent consensus conference on hyperbaric medicine is based on an initial compression of 2.8 or 4 absolute atmospheres (atm abs), without being able to choose between these two tables in the absence of sufficient scientific data (7).

At the Department of Diving and Hyperbaric Medicine of the Sainte Anne Military Hospital, Toulon, France, the initial compression of spinal cord DCS was previously based on a 4 atm abs table (Figure 1), using 50% Heliox (50% oxygen and 50% helium) and oxygen as the breathing gas. Currently, the preferred oxygen tables are at 2.8 atm abs, i.e., tables equivalent to US Navy tables 5 or 6 (Figure 1). The use of these tables at lower pressure levels allows the use of pure oxygen throughout most of the table.

After this initial recompression, additional tables at 2.5 atm abs are often performed if clinical symptoms persist. In recent years, protocols have evolved with the introduction of additional sessions in the first 48 h for the most severe patients. Additional Heliox tables at 4 atm abs have been used and, more recently, additional Heliox tables at 2.8 atm abs have been performed (Figure 2). The aim of these additional Heliox sessions is to benefit from a possible neuroprotective effect of helium (8), which would improve neurological recovery (9).

For severe symptomatic cases, in addition to hyperbaric treatment, a lidocaine protocol has been implemented for its neuroprotective effects in aeroembolic stroke, although its efficacy remains controversial (10–12). The protocol consists of a continuous infusion at an antiarrhythmic dose with continuous cardiac rhythm monitoring.

Prolonged treatment with fluoxetine for a period of 3–6 months is currently proposed not only for its antidepressant effect, but also to stimulate brain plasticity and promote functional recovery in animal models of DCS (13, 14).

Therefore, it seemed important to us to compare these hyperbaric and medical treatments in order to optimize the treatment of this pathology, which can cause significant disability.

The first objective of this study is to compare the effectiveness of initial recompression tables at 2.8 vs. 4 atm abs on the outcome of spinal cord DCS in a series of injured divers with severity criteria during the first 24 h.

The secondary objective is to evaluate the benefit of medical therapy associated with recompression and the value of supplemental hyperbaric tables ≤2.8 vs. 4 atm abs performed in the first 48 h.

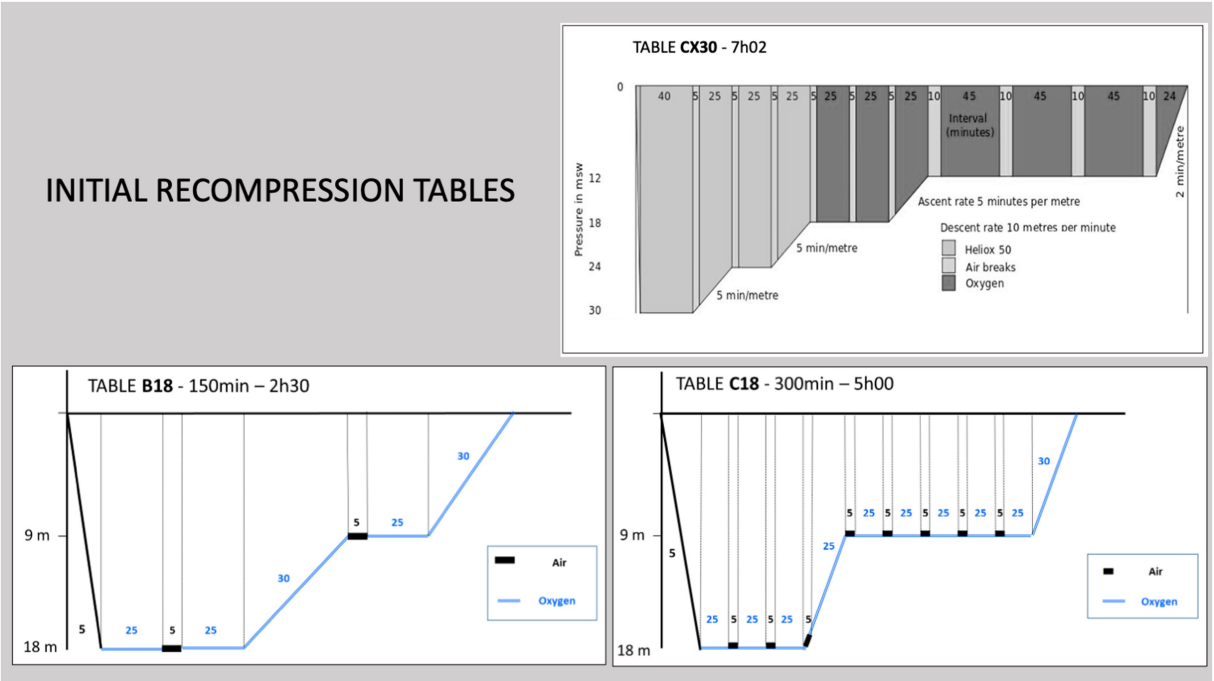

FIGURE 1
Initial recompression tables. When the injured diver had a MEDSUBHYP score of initial severity ≤7, a B18 table was performed, whereas a C18 table or Comex 30 table was used for an initial score > 7.

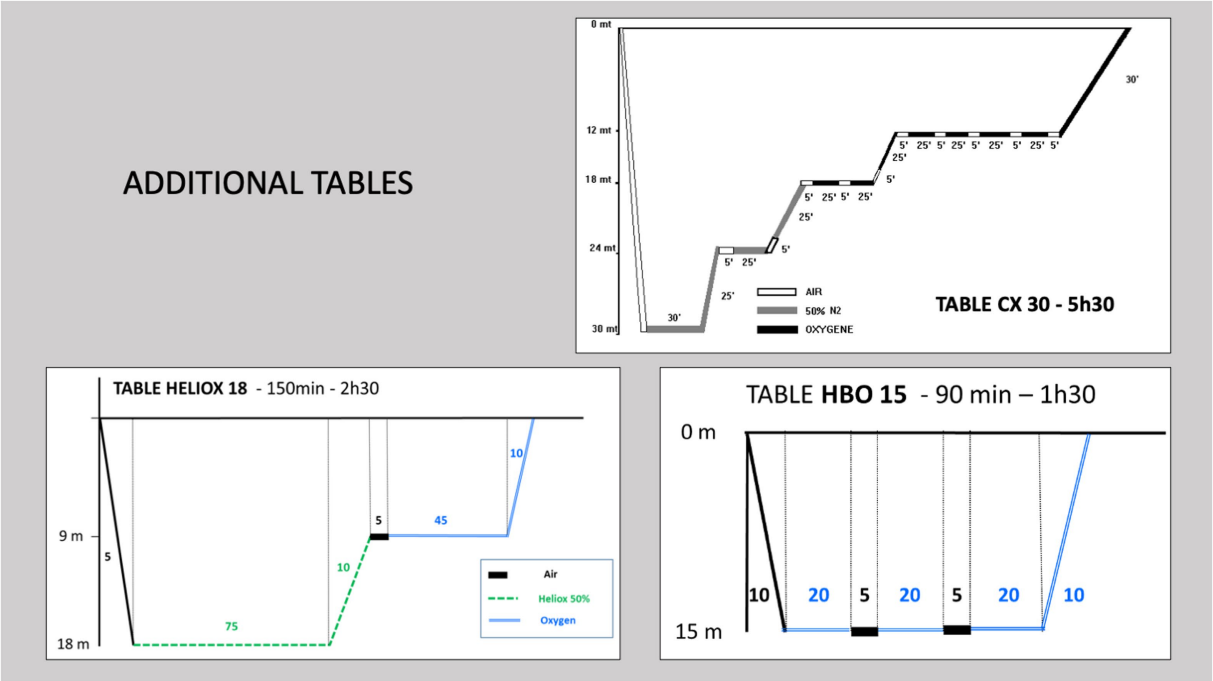

FIGURE 2
Additional recompression tables. Heliox tables at 18 m (2.8 atm abs) and 30 m (4 atm abs) or 100% O2 tables were performed in the first 24–48 h after the initial table followed by consolidation sessions with daily 100% O2 tables at 15 m (2.5 atm abs).

Methods

Inclusion criteria

All patients admitted to the hyperbaric center of the Sainte-Anne military hospital, Toulon, France, between 2010 and 2020 were included in the study if they presented with unilateral or bilateral neurological signs affecting the upper and/or lower limbs or with sphincter impairment within the first 24 h. After reviewing all records, we excluded patients with only subjective signs and symptoms suggestive of brain injury, including cognitive signs consistent with a diagnosis of cerebral DCS or cerebral aeroembolism secondary to pulmonary barotrauma.

Data collection

At the time of patient presentation, the diving medicine specialists at the hyperbaric centre systematically completed a DCS database form with the following information:

- Dive: total dive time, maximum depth, dive level, gas mix (air, nitrox or trimix), equipment (open circuit or rebreather), repetitive dives (dives made in the previous 6 h), procedural errors (rapid ascent, omission of decompression stops).
- Time to recompression (from onset of symptoms to first recompression).
- Clinical data: age, sex, history of DCS and clinical status on admission, assessed by the French Society of Diving and Hyperbaric Medicine score (MEDSUBHYP score), which combines several of these clinical data (Table 1) and allows estimation of the initial severity of neurological DCS (4, 15). Clinical deterioration in the first 24 h after initial recompression was also analysed, independent of clinical status on admission.
- Hyperbaric treatments:
  - initial tables at 2.8 atm abs (O2 100%) or 4 atm abs (Heliox 50% and O2 100%);
  - additional tables within the first 48 h ≤ 2.8 atm abs (O2 100% 2.5 atm abs or Heliox 50% 2.8 atm abs) or 4 atm abs (Heliox); and
  - consolidation tables (O2 100% 2.5 atm abs).

The choice of initial recompression was generally based on initial clinical severity. If the casualty had a MEDSUBHYP score ≤ 7, recompression was generally performed at 2.8 atm abs (O2 100%) for 2 h 30 min (equivalent to US Navy table 5). If the initial score was >7, a procedure at 2.8 atm abs (O2 100%) for 5 h (equivalent to US Navy table 6) or 4 atm abs for 7 h (equivalent to Comex 30 table) was preferred. The ratio of O2 partial pressure equal to 2.8 atm to total table duration is 0.33 for the short table at 2.8 atm, 0.25 for the long table at 2.8 atm and 0.12 for the table at 4 atm abs (Figure 1).

However, over this 10-year period, adherence to this protocol may have varied from practitioner to practitioner, depending on the analysis and habits of each hyperbaric physician.

In the event of deterioration or lack of efficacy of the initial recompression, additional tables were performed within the first 48 h using Heliox tables at 4 atm abs (with one to two sessions), Heliox tables at 2.8 atm abs (two sessions) or oxygen tables (O2 100%, one session/day) at 2.5 atm abs (Figure 2). Supplementary O2 tables for 90 min at 2.5 atm abs (one session/day) were performed on

TABLE 1 The MEDSUBHYP score of initial clinical severity and its numerical weighting.

		0	1	2	3	4	5	6
Age > 42	*No*	X						
	Yes		X					
Back pain	*No*	X						
	Yes		X					
Clinical course before recompression	*Better*	X						
	Stable				X			
	Worse						X	
Objective sensory deficit	*No*	X						
	Yes					X		
Motor impairment	*None*	X						
	Paresis					X		
	Paraplegia						X	
Bladder dysfunction	*No*	X						
	Yes							X

the following days in case of residual symptoms until recovery allowed discharge or clinical stabilisation followed by transfer to a rehabilitation centre. The decision to discontinue or continue sessions was subject to peer review by the physicians at the hyperbaric centre.

- Means of evacuation: helicopter or by road.
- Prehospital treatment: which almost systematically includes normobaric oxygen, hydration (*per os* or by vascular filling) and oral administration of aspirin (250 mg);
- Hospital therapy: vascular filling (infusion of isotonic salt serum, 1 L/5 h), corticosteroids (methylprednisone, intravenous, 1 mg/kg), lidocaine (intravenous infusion, 2 mg/min for 36 h) and fluoxetine (*per os*, 20 mg/d.).

The primary end point was the clinical status of the patient at discharge from the hyperbaric centre, as assessed by the modified Japanese Orthopaedic Association (mJOA) score (16). This score allows a quantification on a total of 17 points. The lower the score, the more severe the deficits; a score = 16 or 17 indicates normal function (Table 2).

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (version 2020). Based on the distribution of the data, continuous values were expressed as mean ± SD and/or median ± interquartile range. There was no *a priori* sample calculation; all patients available in the database were included in the study. For clinical relevance and ease of interpretation, we decided to dichotomize the outcome variable as the presence or absence of sequelae at discharge, as assessed by a mJOA score < or ≥ 16.

The variables available in the database were integrated into a multiple correspondence analysis (MCA), supplemented by a selection of relevant variables based on the literature review. MCA was used to reduce dimensions by identifying collinearity and redundancy between variables.

A univariate analysis was performed to identify predictors of outcomes, using the $\chi 2$ test for categorical variables with Yates correction or the Fisher test for small numbers. For quantitative variables with a normal distribution according to the Agostino & Pearson test, the *t*-test was used. Quantitative variables that did not follow a normal distribution were transformed into qualitative variables by determining discriminatory thresholds through receiver operating curve (ROC) analysis. Variables with a *p*-value ≤0.20 were retained for multivariate analysis with backward elimination logistic regression to control for potential confounders and to identify independent predictors of outcomes. A *p*-value <0.05 was considered significant, and ORs with 95% CIs were reported.

Additional analyses were performed to compare the effect of initial tables on the level of sequelae as a function of initial severity. A two-way analysis of covariance (ANCOVA) was used. The hypotheses of homogeneity of regression lines and homogeneity of variances were tested. Because the assumption of residual normality was not met, the mJoA score was normalized. The Yeo-Johnson transformation with parameter $\lambda = 3.69$ was used, with a Pearson normality statistic of $p = 2.515$.

We also compared mJOA sequelae scores and number of additional HBO sessions performed between tables at 2.8 and 4 atm abs by Kruskal-Wallis test in patients with an initial MEDSUBHYP score > 7 to obtain groups of homogeneous initial clinical severity. In this subgroup of patients with initial severity >7, we also looked for correlations (Spearman), (1) to try to determine whether the ratio of oxygen partial pressure equal to 2.8 atm over total table time could be correlated with the level of sequelae (mJOA score), and (2) whether the total cumulative dose of oxygen delivered (based on the calculation of unit pulmonary toxic dose—UPTD) could also be correlated with the level of sequelae.

In addition, to compare the short and long initial tables at 2.8 atm abs, we performed an analysis in the subgroup with the MEDSUBHYP score interval common to these two initial tables, i.e., >7 and < 19.

TABLE 2 Spinal cord injury assessment score called "modified Japanese Orthopedic Association—mJOA Score" and its numerical weighting.

Criterion	Points
I. Upper extremity motor function	
Unable to feed oneself	0
Unable to use knife and fork, able to eat with a spoon	1
Able to use knife and fork with much difficulty	2
Able to use knife and fork with slight difficulty	3
No disability	4
II. Lower extremity motor function	
Unable to walk	0
Can walk on flat floor with walking aid	1
Can walk up and/or down stairs with handrail	2
Lack of stability and smooth gait	3
No disability	4
III. Sensory function	
A. Upper extremity	
Apparent sensory loss	0
Minimal sensory loss	1
Normal function	2
B. Trunk (same as A)	
C. Lower extremity (same as A)	
IV. Bladder function	
Complete retention	0
Severe dysfunction	1
Mild dysfunction	2
Normal function	3
Total score	**0–17**

Further analyses were performed to evaluate the effect of lidocaine and to compare additional tables at 2.8 (Heliox 50%) vs. 2.5 (100% O2) atm performed in the first 48 h in patients with comparable clinical severity after first recompression by calculating the MEDSUBHYP score at 24 h.

Results

Selection of variables

Multiple Correspondence Analysis (MCA) was performed on all variables available in the database. The axes F1 (10.8%) and F2 (8.3%) are the most relevant to explain for the model: the variables "number of additional HBO sessions" and "mJOA score" contribute significantly to the construction of F1 and the "MEDSUBHYP score" to the construction of F2.

Prehospital variables including means of evacuation and prehospital treatment (normobaric oxygen, hydration, and oral aspirin) were not included as variables in this study. In fact, these variables have recently been the subject of specific analysis and have shown no influence on the level of neurological sequelae in our center (15). The same is true for diving variables, such as diving level, gas and apparatus.

The hospital treatment variables (corticosteroids and vascular filling) were not included in the analysis because they contributed little to the model and were otherwise administered to more than 90% of the population.

The variable "number of additional HBO sessions", which is relevant to the description of the model, is strongly correlated with the level of sequelae (mJOAS). To limit the redundancy of the treatment endpoint, we removed this variable from the analysis, as it is less relevant than the estimation of the level of sequelae according to the mJOAS (15). On the other hand, we included the variable "additional tables in the first 48 h" in the analysis to investigate the specific effect of 2.5, 2.8 or 4 atm abs tables performed in the first 48 h.

Finally, patient characteristics (age, gender, history of DCS), clinical parameters (MEDSUBHYP score), dive parameters (duration, maximum depth, repetitive dives) and the different medical or hyperbaric therapies performed at the hyperbaric center were analyzed as predictor variables and included in the univariate analysis (Table 3).

General description

As described in the flowchart (Figure 3), we included 102 patients, the majority of whom were male, 82 males (80%) and 20 females (20%) with a median age of 52 ± 16 years. 12 patients (12%) had a history of DCS. The median maximum depth was 41 m ± 13 m with a mean dive time of 34 ± 12 min. 17 accidents (17%) occurred during repetitive dives. Procedural errors occurred in only 5 divers (5%) and were not included in the analysis due to their small proportion. The median MEDSUBHYP score was 9 ± 4 and clinical deterioration within 24 h was observed in 49 patients (48%). The median MJOAS at discharge was 16 ± 4 and 47 patients (46%) had neurological sequelae with MJOAS <16. The median time to recompression was 180 ± 147 min with 44 short 2.8 atm abs tables (43%), 35 long 2.8 atm abs tables (34%) and 23 4 atm abs tables (23%). In addition, 98 patients received initial normobaric oxygen therapy (96%), 92 received intravenous hydration (90%), and 102 received aspirin and corticosteroid therapy (100%). After the initial table, 40 patients received a lidocaine protocol (39%) and 36 received a fluoxetine protocol (35%). Finally, 19 patients received additional compression within the first 48 h at 4 atm abs (19%), 36 at 2.8 atm abs (35%), and 47 at 2.5 atm abs (46%).

Univariate and multivariate analysis

Table 3 shows the results of univariate and multivariate analysis.

Initial severity as assessed by the MEDSUBHYP score and clinical deterioration within the first 24 h were associated with the occurrence of sequelae.

In contrast, initial recompression to 2.8 atm abs versus 4 atm tables was significantly associated with a better neurological prognosis at discharge. We found a similar result for the additional ≤2.8 atm abs vs. 4 atm tables.

In addition, treatment with lidocaine was associated with a worse neurological prognosis, whereas there was no significant difference between the groups with or without fluoxetine.

TABLE 3 Results of univariate and multivariate analyses on a series of 102 neurological DCS.

Variables	Sequelae mJOAS <16	No sequelae mJOAS ≥16 (%)	Univariate analysis *p*-value	OR (95% CI)	Multivariate analysis *p*-value	Adj OR (95% CI)
Gender						
Woman	6	14 (70%)		0.43	$p = 0.35$	–
Male	41	41 (50%)	$p = 0.11$	(0.15–1.21)		
History of DCS						
No	40	50 (56%)		–	–	–
Yes	7	5 (42%)	$p = 0.36$			
Age						
≤ 52 y	23	28 (55%)		–	–	–
> 52 y	24	27 (53%)	$p = 0.84$			
Depth (meters)						
(T-test)	43 ± 10	39 ± 10	$p = 0.10$	X	$p = 1.00$	–
Dive (time)						
≤ 34 min	26	27 (51%)		–	–	–
> 34 min	21	28 (65%)	$p = 0.53$			
Repetitive dives						
No	37	48 (56.5%)		–	–	–
Yes	10	7 (41%)	$p = 0.25$			
Initial MEDSUBHYP severity score						
(T-test)	11 ± 6	9 ± 3	**$p = 0.03$**	X	**$p = 0.01$**	0.84 (0.73–0.96)
Clinical worsening in the first 24 h						
No	16	37 (70%)		0.25		0.15
Yes	31	18 (37%)	**$p < 0.001$**	(0.11–0.58)	**$p = 0.004$**	(0.04–0.51)
Time to recompression						
< 194 min	26	33 (56%)		–		–
≥ 194 min	21	22 (51%)	$p = 0.63$		$p = 0.64$	
Initial recompression tables						
2.8 atm abs	31	48 (61%)	**$p = 0.01$**	3.54	**$p = 0.04$**	3.87
4 atm abs	16	7 (30.5%)		(1.37–8.91)		(1.07–15.1)
Additional tables in the first 48 h						
≤ 2.8 atm abs	29	54 (65%)	**$p < 0.001$**	33.5	**$p = 0.01$**	19.5
4 atm abs	18	1 (5%)		(5.06–356)		(2.90–402)
Lidocaine						
Yes	25	15 (37.5%)	**$p = 0.01$**	0.33	$p = 0.49$	–
No	22	40 (64.5%)		(0.14–0.77)		
Fluoxetine						
Yes	10	16 (61.5%)	$p = 0.37$	–	–	–
No	37	39 (51%)				

The level of sequelae was assessed by the modified Japanese Orthopaedic Association Score (mJOAS), i.e., divers presenting sequelae (mJOAS <16) or divers with complete recovery (mJOAS ≥16). A *p*-value <0.05 (in bold) was considered as significant, ORs and adjusted ORs with 95% CIs were reported.

The following variables with a *p*-value ≤0.20 were included in the multivariate analysis: sex, depth, MEDSUBHYP score, clinical deterioration in the first 24 h, initial tables, additional tables, and lidocaine. The variable "time to recompression" was also included as a "forced" variable because of its significant influence found previously (15, 17). After adjustment, a significant association with the occurrence of sequelae was confirmed for initial severity (MEDSUBHYP score) [OR 0.84 (0.73–0.96); $p = 0.01$] and for clinical worsening in the first 24 h [0.15 (0.04–0.51); $p = 0.004$].

Initial recompression at 2.8 atm abs [OR 3.87 (1.07–15.1); $p = 0.04$] and additional compressions ≤2.8 atm abs [OR 19.5 (2.90–402); $p = 0.01$] were significantly associated with improved neurological prognosis.

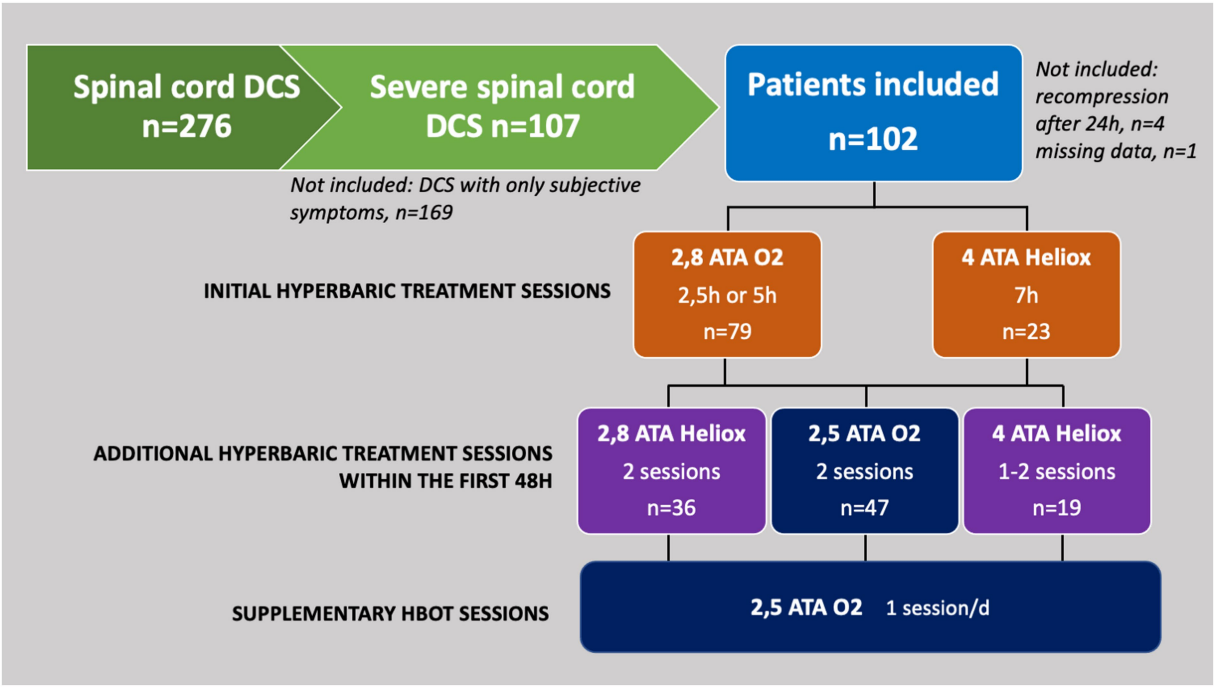

FIGURE 3
Flow chart describing the inclusion of the 102 subjects and the recompression tables performed.

Additional analysis

Effect of initial tables on the level of sequelae as a function of initial severity

Figure 4 shows DCS cases ($n = 102$) treated with initial tables at 2.8 or 4 atm abs according to initial clinical severity (MEDSUBHYP score) and level of sequelae (mJOA score).

Analysis of covariance comparing the regression lines according to the two treatments showed that there was a significant difference ($p = 0.049$) between the 2.8 and 4.0 atm treatments.

Comparison of mJOA sequelae scores between 2.8 and 4 atm abs initial tables

To compare the effect of initial tables in patients with similar initial severity criteria, we selected patients with a MEDSUBHYP score > 7. In this subgroup, MEDSUBHYP scores did not differ with mean values of 12 ± 4 for 2.8 atm abs tables vs. 13 ± 4 for 4 atm abs tables ($p = 0.084$). There were 37% of patients who had sequelae with the 2.8 atm abs tables ($n = 60$) vs. 70% with the 4 atm abs tables ($n = 20$, $p = 0.018$). The mean mJOA scores were significantly different, with a score of 15 ± 3 for the 2.8 atm abs tables indicating a lower level of sequelae than the score of 13 ± 3.5 for the 4 atm abs tables ($p = 0.006$) (Figure 5). In addition, the mean number of additional HBOT sessions required was 6 ± 4 in the group with initial 2.8 atm abs tables versus 9 ± 3 in those with initial 4 atm abs tables ($p = 0.003$).

In this subgroup of patients with an initial severity >7, we found a significant correlation between the ratio of O2 partial pressure equal to 2.8 atm over total table time and the mJOA score, with a correlation coefficient of 0.19 ($p < 0.0001$), indicating a lower level of sequelae when the ratio is higher. However, we found a significant correlation between the cumulative oxygen dose (UPTD) delivered by the initial table and the mJOA score, with a correlation coefficient of −0.48 ($p < 0.0001$), indicating a lower level of sequelae when the oxygen dose is lower.

Comparison of mJOA sequelae scores between short and long initial 2.8 atm abs tables

We compared the efficacy of the long vs. short 2.8 atm abs initial tables on the occurrence of sequelae in patients with the same initial MEDSUBHYP score in the range > 7 and < 19. In this subgroup, MEDSUBHYP scores did not differ with mean values of 11.4 ± 2.5 for both short and long 2.8 atm tables ($p = 0.99$). There were 15% of patients who had sequelae with the short tables ($n = 27$) vs. 41% with the long tables ($n = 27$, $p = 0.066$).The mean mJOA scores were significantly different, with values of 16.5 ± 1 for the short tables indicating a lower level of sequelae than the score of 15 ± 3 for the long tables ($p = 0.018$) (Figure 6).

Subgroup analyses after the first therapeutic table

To allow subgroup analyses after the first therapeutic table, we defined clinical severity after the first hyperbaric treatment by calculating the MEDSUBHYP score 24 h after the start of treatment. This score was associated with an adverse neurological prognosis ($p < 0.0001$) with a severity threshold of ≥13 at 24 h.

Comparison of additional tables performed in the first 48 h at 2.8 or 2.5 atm abs

This severity score allowed us to compare the value of additional tables in the first 48 h in the subgroup of patients with 24-h severity criteria. Only 57% of patients had sequelae with the Heliox 2.8 atm abs tables versus 100% with the Oxygen 2.5 atm abs tables, a result close to statistical significance ($p = 0.06$) (Figure 7).

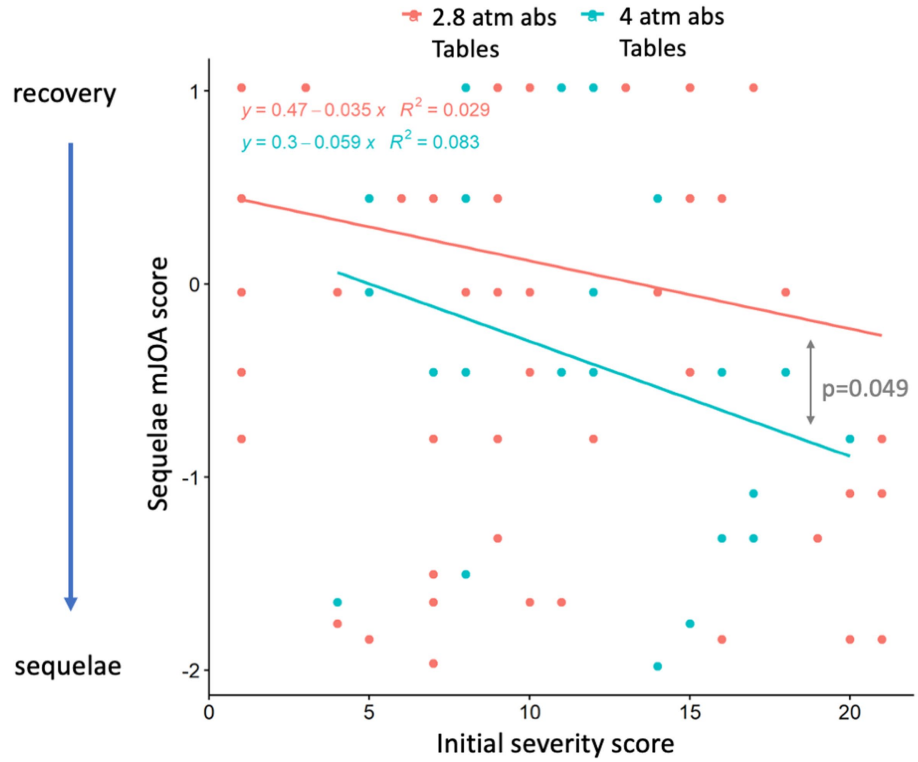

FIGURE 4
Scatter plot of the 102 DCS divers treated with initial tables at 2.8 atm abs (blue) or 4 atm abs (red) as a function of initial clinical severity (MEDSUBHYP score) and level of sequelae (mJOA score). The mJOA score has been normalized according to the MEDSUBHYP score and according to the two treatments (2.8 and 4 atm). Each point represents one or more divers. The analysis of covariance comparing the regression lines according to the two treatments shows a significant difference between the treatment at 2.8 atm and the treatment at 4 atm abs.

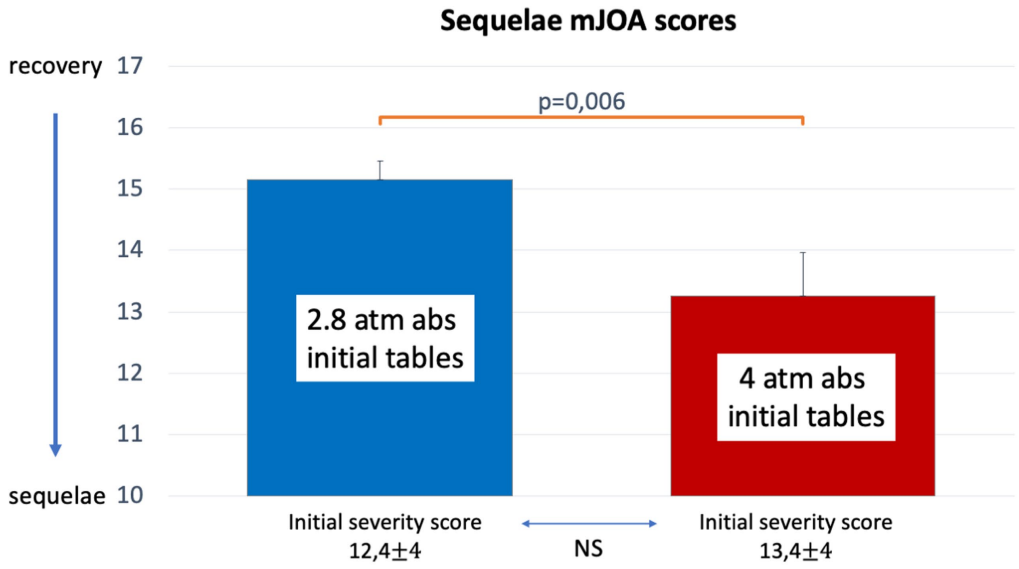

FIGURE 5
Histograms (mean, standard error and *p*-value) showing mJOA score comparisons between initial tables at 2.8 atm abs or 4 atm abs for DCS injuries with an initial MEDSUBHYP score > 7. The mean values with standard deviation of the MEDSUBHYP scores are specified, with no statistical difference between the two groups.

Effect of lidocaine

We also examined the effect of lidocaine treatment in the subgroup of patients who were severe at 24 h. Lidocaine was administered to 72% of patients with clinical severity criteria according to the MEDSUBHYP score at 24 h. 87% of patients treated with lidocaine had sequelae compared to 55% of patients

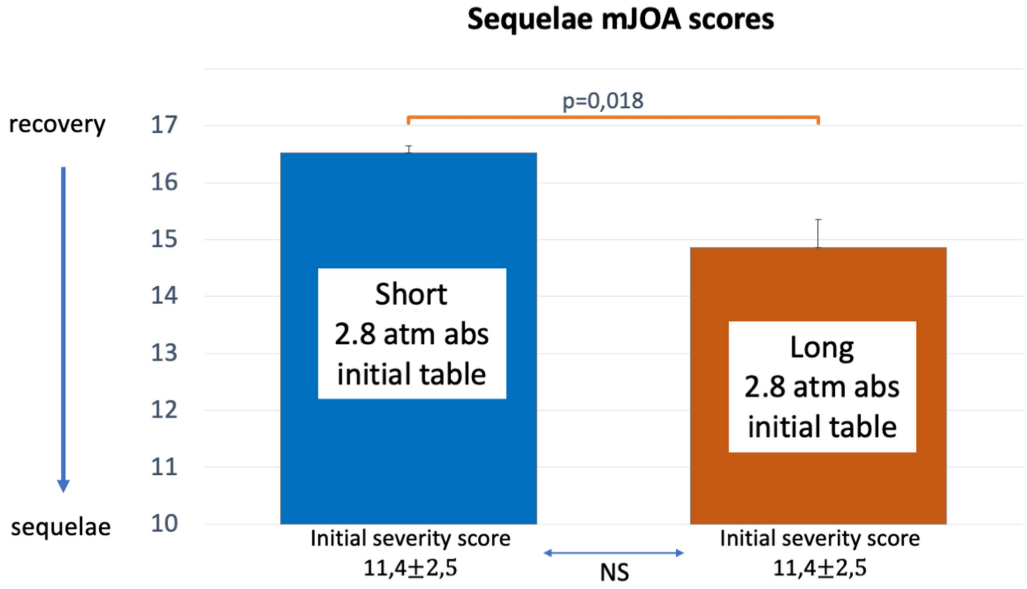

FIGURE 6
Histograms (mean, standard error and *p*-value) showing mJOA score comparisons between Short and Long initial oxygen tables at 2.8 atm abs for DCS injuries with an initial MEDSUBHYP score > 7 and < 19. The mean values with standard deviation of the MEDSUBHYP scores are specified, with no statistical difference between the two groups.

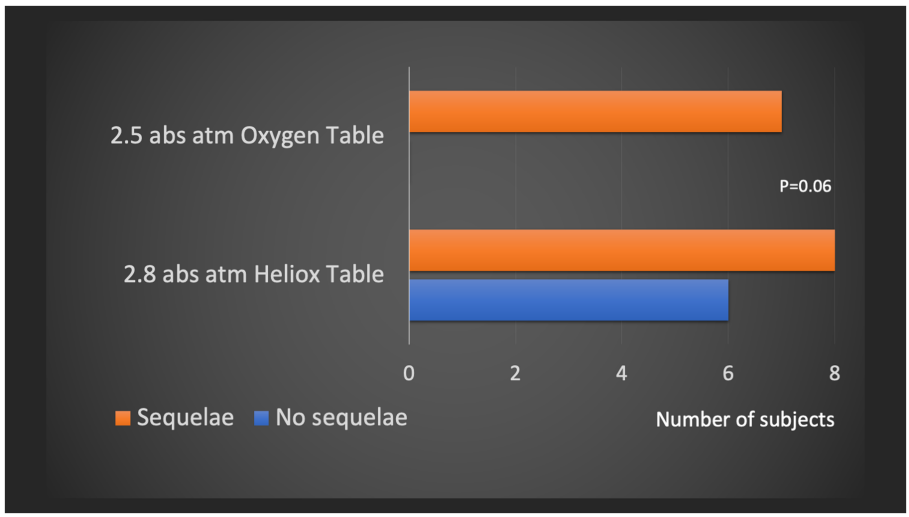

FIGURE 7
Subgroup analysis of patients with equivalent clinical severity at the end of the first recompression. Comparison of the number of sequellar patients (mJOAS <16) according to the additional hyperbaric treatment performed in the first 24–48 h, i.e., the O2 100% table at 2.5 atm abs vs. the Heliox 50% table at 2.8 atm abs.

not treated, but this result was not statistically significant ($p = 0.07$) (Figure 8).

Discussion

Risk factors for neurological sequelae

We found that 46% of divers had neurological sequelae with MJOAS <16 at discharge from the hyperbaric center. Usually, the majority of published series describe a lower rate of sequelae in the order of 20%–30% depending on the study for neurological DCS (4, 15, 18).

This finding may be explained by the fact that our study enrolled patients who presented only with initial severity criteria in the first 24 h. Patients with minor symptoms such as paresthesias during this time window were not included.

Furthermore, despite these inclusion criteria, we found that clinical assessment during initial hospital management using the MEDSUBHYP score remained relevant as a prognostic factor for neurological sequelae. The originality of our study is to highlight the

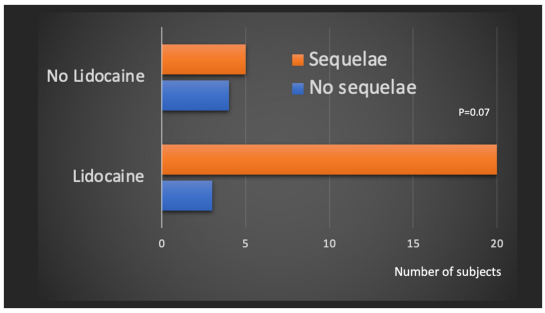

FIGURE 8
Subgroup analysis of patients with equivalent clinical severity at the end of the first table. Comparison of the number of sequelae patients (mJOAS <16) according to the administration or not of a lidocaine infusion.

importance of clinical evolution at 24 h for neurological prognosis at discharge, independent of initial management and time to recompression. After adjustment, this criterion remained significant. This result confirms for the first time the clinical impression of practitioners in hyperbaric centers that a proportion of patients with spinal cord DCS deteriorate after the first recompression. This suggests that there is an activation of biological cascades that are not stopped despite the elimination or reduction of bullae by HBOT. This paradoxical evolution is probably related to the activation of numerous neurotoxic and inflammatory responses triggered by the initial bullous aggression demonstrated in animal models of DCS (19, 20).

Initial recompression

2.8 vs. 4 atm abs tables

For a long time, the symptoms of decompression sickness were thought to be a direct result of the formation of nitrogen bubbles in the body and were assumed to be proportional to depth. Historically, recompression was sought at a depth that would relieve the joint pain associated with decompression sickness. The first therapeutic air recompression tables were constructed between 50% and 100% of maximum pressure before slowly ascending to the surface (21). However, as early as 1878, Paul Bert observed from animal experiments that recompression alone did not eliminate the neurological symptoms of decompression sickness (22). The value of using 100% oxygen tables was applied by Benhke in the early 20th century (23). However, it was not until the end of World War II that the first US Navy oxygen tables were used on a large scale with convincing results. These tables of 100% O2 at 2.8 ATA subsequently became the reference method in the US Navy and many other centers (21). The idea of physically neutralizing the bubbles by applying pressures higher than 2.8 atm abs remains a recurring one, but it is then necessary to dilute the oxygen to limit the risk of hyperoxic crisis. Experimental work on carotid gas embolism in anesthetized dogs suggests that bubble disappearance may be favored by pressures greater than or equal to 4 atm abs (24). However, these results have not been subsequently confirmed. In fact, the work of Leitch et al. showed no benefit in favor of pressures above 2.8 atm abs at the same oxygen partial pressure in animal models of gas embolism or spinal cord injury (25, 26). However, in the second half of the 20th century, numerous deep hyperbaric treatments between 6 and 8 atm abs were performed in different centers in Hawaii (27), Hong Kong (28) or Shanghai (29). However, it is difficult to assess the efficacy of the tables, as most studies only report percentages of improvement ranging from 51% to 94%, without necessarily specifying the clinical condition at admission or at discharge from the hyperbaric center. Comparison of procedures is made even more difficult by the fact that the initial severity and treatment modalities vary enormously between hyperbaric centers (30). In France, two schools of thought have prevailed over the last few decades: the proponents of 4 atm abs Nitrox or Heliox tables for military and professional diving, and the followers of 2.8 atm oxygen tables, comparable to the US Navy T5 and T6 used by in hospitals. In our own hyperbaric center, we have been influenced by these two treatment modalities, with protocols evolving over time from 4 to 2.8 atm abs tables. These changes in management have provided us with a sufficient database to compare these procedures with the results described in this study.

The main result of our study is to show a better neurological recovery when using the initial tables at 2.8 atm abs compared to the tables at 4 atm, and this result persists when we include the potential influence of initial clinical severity as a confounding variable (4, 17).

There are very few comparative studies, an Israeli study (31) attempted to compare the use of the US Navy T6 table with the Comex 30 Heliox table in a series of 33 neurological decompression patients. This study showed no difference in clinical recovery at the end of treatment, but observed a difference in initial clinical severity, which was higher in the group treated with the Comex 30 table.

The supposed superiority of the 4 atm abs table with the Heliox mixture was not confirmed by the Danish experimental work (32) on an animal model (anesthetized rats). This model consisted of injecting bubbles into different tissues (adipose tissue, medullary white matter, muscle and tendon) in order to follow their evolution after decompression and then recompression for therapeutic purposes. Different modalities of recompression were tested at a depth of 2.8 atm abs with inhalation of air, oxygen, Heliox 80% and 50% and at 4 atm abs with Heliox 50%. This work showed that the reduction of bubbles in the white matter of the spinal cord, which is the target tissue for spinal cord DCS, was greater with oxygen recompression at 2.8 atm abs and with 50% Heliox at 4 atm abs, with no significant difference between these two methods.

Recently, a study in an animal model of spinal cord DCS highlighted the value of performing initial tables at 2.8 atm abs at 100% O2 versus higher pressure tables (33). Pigs were subjected to an insult dive at 7 atm abs for 24 min followed by rapid decompression. 61 pigs that developed neurological DCS were randomized to one of four U.S. Navy treatment tables: T6, T6A-air (21% oxygen, 79% nitrogen), T6A-nitrox (50% oxygen, 50% nitrogen), and T6A-heliox (50% oxygen, 50% helium). The authors found no significant differences among the four treatment groups. However, although the trends were not statistically significant, the T6-treated animals had the lowest rates of functional deficits and the least amount of spinal cord injury.

Our results are in line with this experimental study, suggesting a better clinical efficacy of the initial tables at 2.8 atm abs compared to the deeper tables at 4 atm abs. The efficacy of the 2.8 atm abs tables

could possibly be explained by a threshold pressure level above which the effect on bubble compression does not bring any objectifiable gain, with the addition of better denitrogenation during the 2.8 atm abs tables using only 100% oxygen.

The results of our study may also suggest that there is no clinical benefit to this increase in pressure up to 4 atm abs, with a theoretical effect of pressure on bubbles that remains small compared to the reduction in bubble diameter or volume for these recompression levels between 2.8 and 4 atm abs (21).

Another hypothesis is that a high percentage of 100% O2 may not only accelerate the washout of the remaining inert gas in the tissue, but also allow the O2 to equilibrate with the inert gas in the bubble. In a second step, the O2 in the bubble is expected to diffuse into tissues with a lower O2 pressure. Metabolic consumption of O2 could thus contribute to the disappearance or reduction of these residual oxygenated bubbles by counter-diffusion (34).

Due to the risk of neurotoxicity of oxygen, increasing the pressure above 2.8 atm abs implies the use of helium or nitrogen-based diluent mixtures. In this case, the counter-diffusion effect of O2 is less pronounced and the diluent mixtures have a negative effect, contributing to a temporary increase in the inert gas load.

Taking these assumptions into account, the time spent delivering oxygen at high partial pressure, i.e., 2.8 atm (in line with accepted neurological toxicity limits for hyperbaric treatment), should have a greater therapeutic impact in this context than time spent at lower partial pressure levels. To support this hypothesis, we compared the ratio of O2 partial pressure at 2.8 atm over the total duration of the initial tables, looking for a correlation with the level of sequelae. It turns out that the higher the ratio, the lower the level of sequelae, with the best ratio for the short O2 table at 2.8 atm. However, the beneficial effect of a high partial pressure of oxygen can be detrimental if the cumulative dose of oxygen delivered during the table is too high, increasing the risk of oxygen-related toxicity.

That's why we also considered another point based on the correlation we found between the cumulative oxygen dose (UPTD) delivered by the different initial tables and the sequelae score, indicating lower sequelae at lower oxygen doses. Several methods of determining pulmonary oxygen toxicity have been studied. One of the best known is the Unit Pulmonary Toxic Dose (UPTD), which is based on the decrease in vital capacity after dry hyperbaric exposure in resting subjects. The threshold of 615 UPTD is usually considered a safe daily limit for dry hyperbaric exposure. The UPTD is also used to compare therapy tables in terms of cumulative O2 dose delivered (35). The Comex 30 table was found to have the highest oxygen dose (923 UPTD), compared to the long (703 UPTD) or short (377 UPTD) 2.8 atm abs tables in our study. Indeed, the Comex 30 table has recently been shown to expose patients to oxygen-related pulmonary toxicity, manifested by both transient respiratory symptoms and elevated markers of pulmonary inflammation (35).

The onset of this pulmonary inflammatory state may be one of the factors explaining the reduced efficacy of these tables. In fact, during the crucial first 24 h, the organism, initially attacked by the formation of post-decompression bubbles, reacts by activating several cascades that generate a generalized immuno-inflammatory state.

In this context, an increase in the inflammatory state associated with hyperbaric treatment must be avoided at all costs. For this reason, 2.8 atm abs tables, similar to USN T5 or T6 tables, shorter than Comex 30, seem preferable. In addition, these 2.8 abs atm oxygen tables are able to inhibit leukocyte adhesion and reperfusion injury (36), which is not documented with the Heliox 50% table at 4 abs atm.

Taking the results of the two correlations together, it appears that the benefit of tables in the treatment of DCS corresponds to a balance between the ability to deliver O2 at a high partial pressure, i.e., 2.8 atm, and a limited exposure time in order to deliver a cumulative dose of oxygen that does not lead to pulmonary inflammation associated with O2 toxicity.

Short vs. long 2.8 atm abs tables

Regarding the duration of recompression in a cohort of patients with comparable initial severity, 15% of patients had sequelae with a short 2.8 atm table versus 41% with a long table, with a significant difference in mJOA scores.

Short tables at 2.8 atm abs are generally not recommended for the treatment of severe neurological DCS, yet their supposedly lower efficacy remains to be demonstrated.

However, the treatment of DCS with a single short table at 2.8 atm abs has long been developed in the United States for the hyperbaric chamber of construction sites. These short tables (2.5 h) at 2.8 atm abs seem to give good results in the published series. Hart et al. (37) note 79%–95% recovery depending on the clinical form for 77 DCS treated with the Hart-Kindwall table. Cianci & Slade (38) found 97.5% recovery in a series of 140 neurological DCS with cerebral signs, including cognitive signs, recompressed in the 48 h.

Recently, a prospective randomized Australian study concluded that a short table at 2.8 atm abs was more effective than the USNT6 table (39). However, this study only involved mild DCS without neurological impairment.

Furthermore, as previously discussed, these short tables at 2.8 atm abs are likely to generate less pulmonary inflammation linked to pulmonary oxygen toxicity than longer tables (35).

From our point of view, determining the optimal duration of the initial table at 2.8 atm abs for the management of neurological DCS is an issue that needs to be studied, particularly for the most severely affected patients.

Additional hyperbaric treatment in the first 48 h

Our study suggests that additional tables at 4 atm abs may be less effective than tables at 2.8 atm abs (Heliox 50%) or 2.5 atm abs (O2 100%).

After initial recompression, a certain number of patients do not recover, and we thought it appropriate to provide additional specific hyperbaric treatment within the first 24–48 h, based on the hypothesis that this period corresponds to the peak of post-ischemic phenomena after initial reperfusion. For example, it has been emphasized that deleterious processes such as leukocyte activation are observed in DCS (19, 20). Therefore, in addition to the physical effects on bubbles and oxygenation, HBOT sessions may also be of interest in inhibiting leukocyte adhesion or other anti-inflammatory processes (36).

Furthermore, we believe that the use of helium in combination with HBOT may also activate complementary neuroprotective effects. Therefore, over the years we have developed different protocols for additional tables with 50% Heliox at 4 atm abs and more recently at 2.8 atm. However, our study suggests that additional tables at 4 atm may be less effective than tables at 2.8 atm (Heliox 50%) or 2.5 atm (O2 100%). This lower effectiveness of 4 atm tables may also be related to the previously mentioned problem of inflammation associated with pulmonary O2 toxicity, which favours the use of secondary tables with lower partial pressures after the first table (35).

Currently, a complementary recompression protocol with a lower partial pressure of O2, including Heliox 50% tables at 2.8 atm, is used in the event of an unsatisfactory neurological outcome of DCS. The neuroprotective effects of helium have not been clearly identified and may be mediated by induced hypothermia (40), antithrombotic effects (41), inhibition of apoptosis (42) and stimulation of neoangiogenesis (43). Our study does not formally conclude that these additional Heliox sessions are more effective than 100% O2 HBOT sessions. However, subgroup analysis shows that for patients with equivalent 24-h severity criteria, 57% of patients in the group using the Heliox 2.8 atm abs tables had sequelae, whereas 100% of patients in the group using the 2.5 atm abs tables had sequelae, a result approaching statistical significance ($p = 0.06$).

The study by Drewry and Gorman (44) also suggests that the use of 2.8 atm abs Heliox 50% vs. 2.5 atm abs O2 100% HBOT tables may reduce the number of patients requiring additional HBOT sessions used as an efficacy criterion (9 subjects/25 vs. 20/31). However, these partial results, based on a small series (only abstracts have been published), should be confirmed.

It would be interesting to carry out a prospective study comparing these complementary sessions with Heliox or O2 100%, which would allow us to know if the use of helium really brings a benefit. Other gases could also be good candidates to optimise the neutralisation of neurotoxic cascades with anti-NMDA agents such as xenon or argon in combination with HBOT (42, 45–49).

Drug therapies

Very few studies have been conducted to evaluate the efficacy of drug treatments in DCS in humans. Only one randomized controlled trial in humans suggests the value of using a non-steroidal anti-inflammatory drug, tenoxicam, based on the number of additional HBOT sessions performed as a primary endpoint (9).

In fact, drug prescription in hyperbaric centers is mainly based on pathophysiological knowledge and animal DCS model studies (11, 21). Our study did not allow us to analyse the effects of the treatments commonly used in our centre, namely vascular filling with isotonic saline and the use of low-dose intravenous corticosteroids.

However, we were able to assess the effect of lidocaine, which appeared to be associated with a higher risk of neurological sequelae in the univariate analysis, although this was not confirmed after adjustment. In an additional analysis, we found that lidocaine was more frequently used in patients with high clinical severity at 24 h (72%), with a still very high rate of sequelae (87%) despite treatment. Its cardiac and neurological toxicity, narrow therapeutic margin and lack of efficacy on neurological prognosis highlighted in our study are arguments against its continued use in severe spinal cord DCS.

Fluoxetine is a selective serotonin reuptake inhibitor that is prescribed for an extended period of 3 to 6 months. This antidepressant treatment contributes to the psychological support of sequelae patients who are secondarily transferred to rehabilitation centers for long months. We also prescribe this treatment for its neuroprotective effects reported in the DCS animal model (13, 14), with an improvement in clinical recovery in ischemic stroke reported in humans (50). This molecule is used not only for its antidepressant effect, but also for anti-inflammatory purposes via the interleukin pathway acting on the central nervous system (13). Our study found no effect of fluoxetine on neurological prognosis at discharge from the hyperbaric center. However, we do not have information on the extent of long-term neurological sequelae. To determine the true effect of fluoxetine, we would need to conduct a study evaluating neurological recovery at least 6 months after hyperbaric treatment.

Limitations

The limits of our study lies in its retrospective and mono-centric nature as well as in the small number of patients tested due to the low prevalence of this pathology.

Different protocols have indeed been applied over this 10-year period within the hyperbaric medicine service. These different management modalities can be considered as the main limitation of this study, however it is thanks to this diversity of practice that we were able to compare the different drug treatments and the hyperbaric tables performed, taking into account the main variables that would have influenced the comparison such as the initial clinical severity.

To investigate potential biases related to the retrospective nature of the study, we performed additional statistical analyses, particularly to account for initial clinical severity, which may influence the level of sequelae and potentially the effect of hyperbaric treatment. Although the result was not statistically significantly different, the mean MEDSUBHYP scores for the 2.8 atm abs tables were lower than those for the 4 atm abs tables. This is explained by the inclusion of a certain number of patients whose initial severity on admission was lower, but who deteriorated within the first 24 h. To account for this possible source of bias, we performed an overall analysis of covariance as well as subgroup analyses of patients with high and comparable initial severity. The results of these complementary analyses confirm that the initial tables at 2.8 atm abs are associated with better clinical recovery than the tables at 4 atm abs, and the short tables at 2.8 atm are also associated with better results than the long tables at 2.8 atm. Overall, our multivariate analysis accounts for potential sources of bias related not only to initial clinical severity, but also to time to recompression and deterioration within the first 24 h.

We did not examine the separate effects of hyperbaric treatment variables such as pressure level *per se*, oxygen partial pressure level, or the specific effects of gas mixtures. As these variables are related to the therapeutic tables chosen, specific studies would be required to

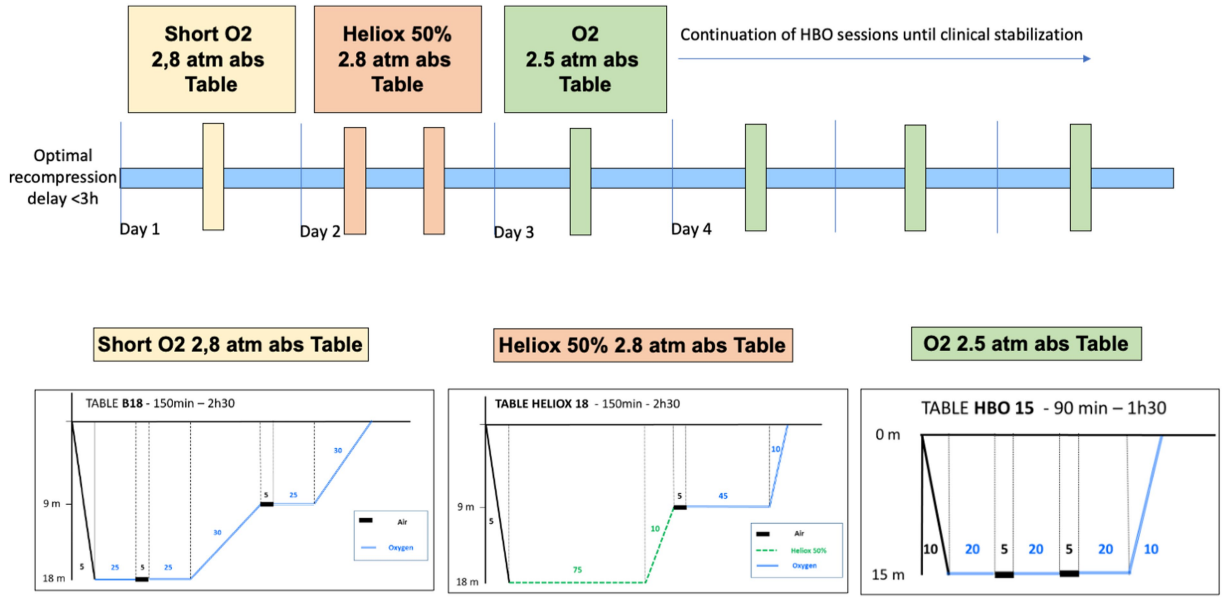

FIGURE 9
Proposed hyperbaric therapeutic management of spinal cord DCS based on results suggested by this study.

disentangle the effects of these different variables. Our results therefore apply only to the test procedures described in this document, and the discussion of hypothesized mechanisms must also take into account this degree of uncertainty associated with the interrelated effect of these variables.

We are aware that patients with brain damage may also present with neurological signs similar to those of spinal cord DCS, but in order to select as homogeneous a group of patients as possible, we preferred to restrict the inclusion criteria and not to retain documented brain damage.

In this study, we do not have an additional assessment several months after hospitalization to determine neurological clinical status. We did not choose the Rankin Score, which is often used to assess neurological sequelae. In addition, our decision to dichotomize the outcome may have resulted in the loss of some information from the data set. However, we felt that it was clinically preferable to maintain this separation between patients with significant improvement or recovery and those with sequelae requiring rehabilitation center care.

Conclusion

Our study suggests that initial recompression of patients with neurological deficits related to spinal cord DCS should be performed with oxygen tables at 2.8 atm abs rather than at 4 atm (Heliox 50% Comex 30). Optimal treatment appears to be a balance between the ability to deliver O2 at a high partial pressure of 2.8 atm and a limited exposure time that does not result in the pulmonary inflammation associated with O2 toxicity. Short tables at 2.8 atm (equivalent to USNT5) appear to provide a better clinical response than long tables (equivalent to USNT6), with the limitation that the comparison could not be made for the most severe patients.

If there is no response after the first table, our study also suggests one or two additional sessions of Heliox 50% tables at 2.8 atm within the first 24–48 h (Figure 9).

Furthermore, our study shows that the risk of sequelae is not only related to the initial severity, but also to the clinical deterioration in the first 24 h, suggesting the activation of biological cascades that are not stopped by the initial recompression. We believe that the application of hyperbaric treatment must take into account this immuno-inflammatory state by trying to determine the appropriate dose, depending not only on the pressure and duration of treatment, but also on the partial pressure of oxygen and the specific potential neuroprotective effect of certain therapeutic gases, such as helium. Clearly, studies are needed to measure the immuno-inflammatory responses of patients and to understand the effects of hyperbaric therapy on these secondary processes associated with decompression sickness.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Commission de validation des études cliniques HIA Sainte Anne Toulon. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because study only based on retrospective data.

Author contributions

BS and J-EB: conception and design of the study, analysis and interpretation of the data, and drafting and revising the manuscript. BS, RR, HL, JM, AD, LD, PL, SM, EG, NV, and J-EB: interpretation of the data and revising the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Advances in hyperbaric oxygen to promote immunotherapy through modulation of the tumor microenvironment

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Hyperbaric oxygen therapy is a relatively safe treatment method that has been used for a long time in the clinic. It has been proven that it can enhance the sensitivity of radiotherapy and photodynamic therapy for cancer. However, there are few studies on hyperbaric oxygen and immunotherapy. In this article, we summarize that hyperbaric oxygen therapy regulates the tumor microenvironment through various pathways such as improving tumor hypoxia, targeting hypoxia-inducing factors, and generating reactive oxygen species. The change in the tumor microenvironment ultimately affects the curative effect of immunotherapy. Therefore, hyperbaric oxygen can influence immunotherapy by regulating the tumor microenvironment, providing a direction for the future development of immunotherapy.

KEYWORDS

immunotherapy, tumor microenvironment, hyperbaric oxygen, hypoxia-inducing factors 1α, reactive oxygen species

Introduction

The high morbidity and mortality rate of cancer seriously affect people's health. The treatment of tumors mainly includes surgical resection, radiotherapy, chemotherapy, targeted therapy, immune checkpoint inhibition, and so on (1). Immunotherapy is one of the successful methods. Its mechanism is to block the immune checkpoint expressed by tumor cells and enhance the killing effect of T cells (2). Immune checkpoint blockers (ICBs) mainly act on immunosuppressive targets, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), or block immune checkpoint-related ligands, such as programmed cell death ligand 1 (PD-L1). Therefore, CTLA-4 antibody and PD-1/PD-L1 antibody are the main immune checkpoint inhibitors in clinical applications (3, 4). Although PD-1/PD-L1 antibodies target two endpoints of the same immune pathway, they are quite different in mechanism of action, clinical efficacy, and drug resistance (5) (Figure 1). In the process of clinical application, immunotherapy

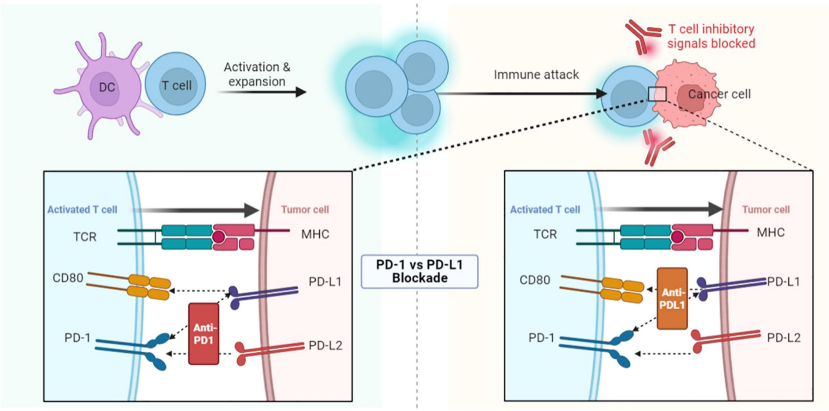

FIGURE 1
PD-1/PD-L1 antibodies target two endpoints of the same immune pathway and therefore have very different mechanisms of action and clinical efficacy. DC, Dendritic cells; TCR, T cell receptor; CD, Cluster of differentiation; PD, Programmed cell death protein; PD-L, Programmed cell death ligand; MHC, Major histocompatibility complex.

has experienced unpredictable primary and acquired drug resistance (6), which has affected its promotion and sustainable application (2, 7). Immunotherapy has brought survival benefits to countless cancer patients since its advent. Therefore, overcoming drug resistance to immunotherapy is particularly important in its long-term development. At present, it has been found that the tumor microenvironment has a certain influence on immunotherapy (8, 9).

Hyperbaric oxygen (HBO) therapy, as a clinical treatment with a certain history, has been widely used in hypoxia and wound healing (10). In recent years, studies have shown that HBO can improve the curative effect of radiotherapy and photodynamic therapy for tumors (11). Whether HBO can promote T cells to enter the tumor core, improve tumor-killing activity and promote immunotherapy is still a problem worthy of study (12). This article will discuss the relationship between HBO and immunotherapy from the tumor microenvironment level, and further clarify the influence of hyperbaric oxygen on immunotherapy.

Tumor microenvironment: (hypoxia, blood vessel, extracellular matrix, hypoxia-inducible factor 1α)

Tumor microenvironment refers to the local biological environment in which solid tumors are located, including cancer cells and their nearby stromal cells (13). In the early stage of tumors, passive diffusion is the main way for cancer cells to transport nutrients. As tumor size increases, insufficient oxygen supply and metabolic waste accumulation will cause hypoxia and acidosis in the tumor microenvironment. The hypoxic tumor microenvironment induces immature neovascularization, which leads to vascular leakage (14). Extracellular matrix (ECM), as an important part of the tumor microenvironment (15), not only provides a physical scaffold for cancer cells but also plays a key role in diffusion and drug resistance.

Hypoxia

Hypoxia can activate hypoxia-inducible factor 1α (HIF1α) (16), which upregulates PD-L1 expression on dendritic cells and cancer cells, leading to immunosuppression (17, 18). Hypoxia also can inhibit the activity of T cells and the antigen-presenting ability of dendritic cells (19, 20). Hypoxia can induce invasive matrix molecules and increase the invasive potential of cancer cells (21). It can also up-regulate the expression of drug-resistant molecules, induce cell cycle arrest, and lead to the insensitivity of cancer cells to radiotherapy and chemotherapy (22).

Myeloid-derived suppressor cells (MDSC) are the largest group of suppressor cells in the tumor microenvironment and are considered the main obstacle to immunotherapy (23). Hypoxia can recruit immature myeloid cells and transform them into MDSC. MDSC can also be recruited by secreting chemokines (24). Hypoxia can also directly combine with PD-L1 to selectively up-regulate MDSC (18). The activation of MDSC can lead to immunosuppression (Figure 2).

Blood vessel

Hypoxia can also induce vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) to destroy the stability of blood vessel walls (25, 26) and induce immature neovascularization. The local high permeability of blood vessels can cause plasma to leak from blood vessels into tumor stroma, which leads to an increase in extravascular hydrostatic pressure (27) and hinders drug transportation. Most anti-cancer drugs exert selective toxicity on cells, so cells that proliferate slowly are usually drug-resistant (28). As the distance from tumor vessels increases, the proliferation of tumor cells decreases gradually, and the concentration of exposed drugs decreases, which eventually leads to drug resistance (29).

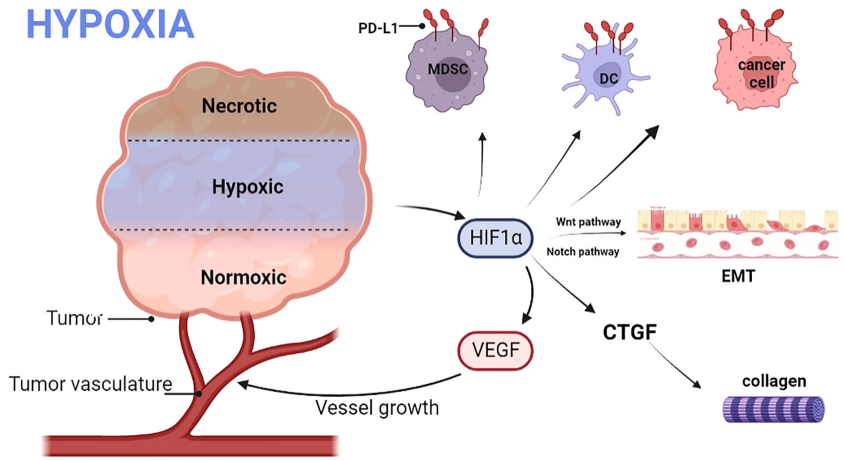

FIGURE 2
Hypoxia promotes the release of HIF, leading to an increase in VEGF, which in turn promotes the growth of tumor vessels. HIF also promotes the expression of PD-L1 on MDSC, DC, and tumor cells; promotes EMT via the Wnt and Notch pathways. Finally, HIF also promotes the production of collagen fibers. DC, Dendritic cells; PD-L, Programmed cell death ligand; MDSC, Myeloid-derived suppressor cells; HIF1α, Hypoxia-inducible factor 1α; EMT, Epithelial-mesenchymal transition; VEGF, Vascular endothelial growth factor; CTGF, Connective tissue growth factor.

Extracellular matrix

ECM is composed of collagen, fibronectin, and elastin, which is an important part of the tumor microenvironment. Hypoxia up-regulates HIF1α, induces connective tissue growth factor (CTGF), and regulates collagen deposition (22). Collagen deposition forms a denser ECM, which promotes the directional migration of cancer cells. ECM derived from anoxic fibroblasts was found to be 3 times stiffer than ECM derived from non-anoxic fibroblasts (30). Therefore, the dense ECM in the tumor microenvironment affects the curative effect of small molecule drugs, let alone the infiltration of Cytotoxic T lymphocyte (CTL) and PD-1 antibody (31).

Hypoxia-inducible factor 1α

HIF1α is the core of hypoxia response (32), and it is also an important regulatory factor for cells to adapt to hypoxia (33–35). Under physiological conditions, HIF1α was easily degraded (36). When the oxygen partial pressure in the body decreases, HIF1α will accumulate (32). HIF1α is pleiotropic, including metabolic adaptation, neovascularization, and metastasis (33, 34).

Epithelial-mesenchymal transition (EMT) is a biological process in which epithelial cells are transformed into mesenchymal phenotypic cells through specific processes (37). HIF1α is the key transcription factor of EMT. Recent studies have shown that HIF1α can induce EMT, which leads to metastasis and poor prognosis of hepatocellular carcinoma (HCC) (38). Long non-coding RNA (lncRNA) can inhibit T cell immune function by affecting regulatory T cell (Treg) and PD-1/PD-L1 immune checkpoints (39). Under hypoxia, HIF1α can target lncRNA to influence immunotherapy. HIF1α activates the expression of PD-L1 by directly binding to the hypoxia response element in the proximal promoter of PD-L1. HIF1α induces VEGF and inhibits dendritic cell maturation (40, 41). VEGF down-regulates T cell function by enhancing PD-L1 expression in dendritic cells (42–44). Therefore, HIF1α may be the key factor of drug resistance in immunotherapy.

In a word, the tumor microenvironment is not only a silent bystander but an active promoter in the process of cancer occurrence (45). Studies have shown that the immune tolerance of tumors can be attributed to the tumor microenvironment of immunosuppression (46). Therefore, targeting the tumor microenvironment can enhance the effect of tumor immunotherapy to a certain extent.

Hyperbaric oxygen

Hyperbaric oxygen therapy is based on nearly 100% pure oxygen (at least 95% oxygen) and increased barometric pressure (47). When the patient inhales 100% oxygen, the extra pressure will increase the dissolved oxygen in plasma and increase the oxygen tissue transport independent of hemoglobin (48, 49). In addition, increased barometric pressure produced by HBO therapy may exert anti-tumor biological activity through gene expression (50). This is an incomparable advantage of HBO over other oxygen delivery methods (31). HBO is often used as the main means to treat carbon monoxide poisoning, decompression sickness (51, 52), and other ischemic and hypoxic diseases. Malignant tumors were once a contraindication of HBO. More and more evidence proves that HBO has a neutral effect on malignant tumors (48, 53). Studies have shown that HBO can reduce drug resistance to chemotherapy and radiotherapy (54). In conclusion, there is no research to prove that HBO promotes cancer recurrence and metastasis so far (48, 55). In some tumor models, HBO can inhibit the proliferation of cancer cells and stimulate the apoptosis of cancer cells (49). Therefore, the role of HBO in malignant tumors needs further study.

Hyperbaric oxygen affects the immune system

HBO therapy has broad-based effects on the immune system in normal individuals and human disease. By observing the antibody reaction of sheep erythrocytes, it was found that HBO had an immunosuppressive effect on normal mice and autoimmune mice. HBO can lead to lymphocyte death through direct oxygen cytotoxicity or endogenous steroid hormones induced by oxidative stress (56). In autoimmune diseases, HBO can selectively eliminate abnormal lymphocyte subsets, showing potential therapeutic effects (57). Shao-Yuan Chen found that HBO can reduce the deposition of immune complexes in the kidney of lupus nephropathy mice and improve the survival rate (58). After HBO exposure, the production of pro-inflammatory cytokines and the level of steady-state RNA in blood-derived monocytes were inhibited (59).

In addition, HBO can also affect immune response by regulating gene expression. Ye Chen analyzed gene expression after exposure to different levels of partial oxygen pressure and found that both independent and overlapping genes were sensitive to increased pressure and/or oxygen (60). After genome-wide microarray analysis of human microvascular endothelial cells, Godman found that up to 8,100 genes were up-regulated or down-regulated within 24 hours after exposure to HBO. The up-regulated genes are mainly growth and repair hormones and anti-inflammatory genes, while the down-regulated genes are mainly pro-inflammatory and apoptotic genes (61). Based on much literature, Paul G. Harch concluded that hyperoxia and/or atmospheric pressure have a wide range of promoting and inhibiting effects on gene expression (50). HBO activates the expression of genes that protect and promote the growth of endothelial cells and enhances the function of endothelial cells. HBO regulates the up-regulation of anti-inflammatory genes and down-regulation of pro-inflammatory genes, thus reducing inflammatory response (61). Therefore, the combination of HBO and immunotherapy may up-regulate immune genes. Finally, gene therapy plays an anti-tumor role.

Hyperbaric oxygen regulates the tumor microenvironment (hypoxia, blood vessels, ECM)

Normobaric hyperoxia, meaning hyperoxia from breathing an increased FiO2 of oxygen at ambient atmospheric pressure. Scholars have found that normobaric hyperoxia can induce apoptosis by regulating the tumor microenvironment. Normobaric hyperoxia can enhance the anti-tumor activity of T cells and natural killer cells (NK), leading to the death of tumor cells (62). Normobaric hyperoxia provides a feasible direction for improving the immunotherapy of cancer. Both HBO and normobaric hyperoxia use oxygen to improve tumor hypoxia. Therefore, the effect of HBO on the tumor microenvironment is worth exploring.

In the mouse HCC tumor model, HBO uses oxygen to oxygenate the tumor, relieve tissue hypoxia and improve the anti-tumor effect of Doxil (22). In the pancreatic cancer model, HIF1α expression decreased after HBO (63–65). Pan Wang found that HBO enhanced the sensitivity of chemotherapy drugs by inhibiting the expression of HIF1α (66). HBO promotes immunotherapy by relieving tissue hypoxia and down-regulating PD-L1 (67).

HBO can promote angiogenesis in patients with traumatic brain injury (68–70). Katarzyna Stępień believes that HBO can be used as an adjuvant in chemotherapy to promote the development of new blood vessels and the transportation of drug molecules (49). In a mouse model inoculated with human epithelial ovarian cancer cells subcutaneously, T Alagoz found that HBO increased the efficacy of cisplatin by inducing angiogenesis (71). Cluster of differentiation (CD) 31, as a mitogenic factor in wound healing, is highly expressed in endothelial cells and related to tumor angiogenesis. Shao-Yuan Chen found that CD31 expression increased significantly 14 and 28 days after HBO treatment. HBO improved tumor angiogenesis but did not increase tumor growth (54). However, in breast cancer (72–74) and glioma models, the diameter and density of tumor peripheral blood vessels decreased significantly after HBO treatment (75). The effect of HBO on tumor vessels may depend on the tumor model, animal species, or other factors. The role of HBO in angiogenesis remains controversial.

Cancer-associated fibroblasts (CAFs) can produce dense ECM, which confines T cells to the matrix and inhibits the anti-tumor immunity of T cells (76). In the mouse pancreatic cancer tumor model, HBO significantly inhibited CAFs (63). After HBO treatment, the transcription and expression of CTGF and type I collagen decreased significantly, and dense ECM was decomposed. HBO can directly consume collagen fibers and fibronectin in ECM, promoting drug transport (63). In a word, HBO consumes the dense ECM around tumor cells through various mechanisms, increases the infiltration of PD-1 antibodies and T cells into tumor parenchyma (31), and promotes the immunotherapy of cancer (Figure 3).

HBO can reduce the number of Treg cells in tumor tissue and alleviate the immunosuppressive microenvironment (31). To sum up, HBO can target the tumor microenvironment to promote cancer immunotherapy.

Hyperbaric oxygen targets HIF1α

In the chronic lymphocytic leukemia (CLL) mouse model, decreasing the expression of HIF1α can increase the survival rate of the CLL mouse model. HIF1α inhibitors can exert toxicity on CLL cells (33). HIF1α inhibitor has a strong anti-tumor function, and combined with ibrutinib can induce cytotoxicity (34). Therefore, targeting HIF1α is a promising therapeutic strategy.

HIF1α mediates the immune escape of various hypoxic solid tumors. Qinghua Wu et al. found that HIF1α inhibitors can reduce the expression of PD-L1 (77). Xing-Chen Ding proved that targeting HIF1α can improve the therapeutic effect of anti-PD-1/PD-L1 in glioma (78). Therefore, blocking PD-L1 and inhibiting

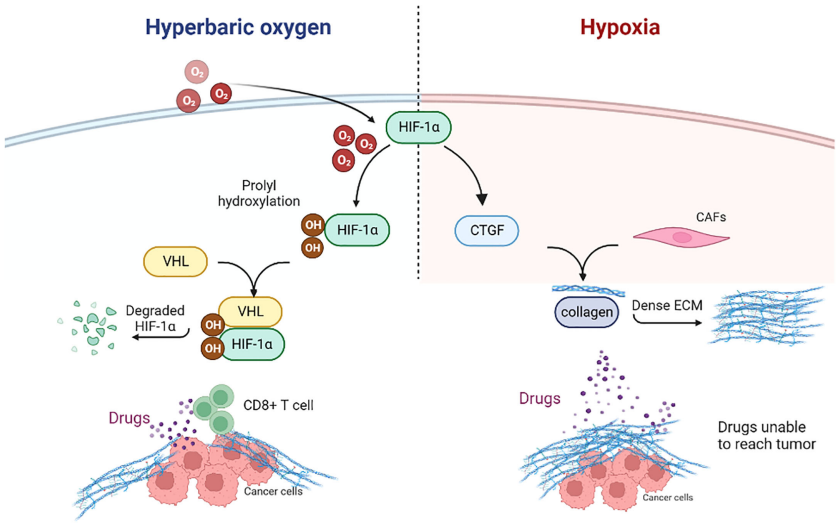

FIGURE 3
Hyperbaric oxygen can decompose dense ECM through various mechanisms. HIF1α, Hypoxia-inducible factor 1α; CTGF, Connective tissue growth factor; CAFs, Cancer-associated fibroblasts; ECM, Extracellular matrix; VHL, Von Hippel Lindau; CD, Cluster of differentiation.

HIF1α is a promising combination therapy (79, 80). Inhibition of HIF1α can release the anti-tumor activity of NK cells (81). Yen-An Tang found that inhibition of HIF1α can reverse chemotherapy resistance caused by tumor microenvironment (82). In a word, the HIF1α pathway plays an important role in the treatment of cancer.

HBO can inhibit HIF1α in tumors. HBO inhibits the Warburg effect, hyperproliferation, and EMT of non-small cell lung cancer cells by down-regulating HIF1α (83). In the glioma model, HBO inhibited HIF1α and improved prognosis (66). HBO can regulate the HIF1α/CTGF/type I collagen pathway (22) and improve dense ECM.

HBO can not only reduce the expression of PD-L1 (67) but also down-regulate HIF1α. Therefore, it has a positive role in promoting immunotherapy.

Hyperbaric oxygen produces ROS

Reactive oxygen species (ROS) is an oxygen-containing molecule that protects and harms cancer cells. An appropriate amount of ROS can regulate biological function and intracellular homeostasis, while an excessive amount of ROS can induce cell death through various mechanisms (84). ROS can act as a signaling molecule and regulate EMT in many ways (85). Many studies have shown that ROS has dual effects on cancer. Therefore, we need to dialectically view the role of ROS in cancer treatment (86).

HBO can produce excessive ROS (87). In the HBO environment, photodynamic therapy can generate a large amount of ROS in hypoxic tumors. At the same time, the fluorescence intensity of HBO-treated cells was significantly higher than that of normal oxygen-treated cells, suggesting the generation of ROS (88).

ROS can be involved in the initiation and metastasis of cancer (85). ROS can also stabilize HIF1a, and cause cancer metastasis and drug resistance (89). In a glioma mouse model, HBO can induce ROS in the thymus, inhibit T cell maturation, leading to immunosuppression, and finally promote the growth of malignant glioma cells (90). There are two types of macrophages, Macrophages1 (M1) is involved in tumor killing, and Macrophages2 (M2) is involved in tumor growth and metastasis (86, 91). In lung cancer and breast cancer models, ROS is necessary for the tumor to acquire the M2 phenotype (92). ROS can promote macrophage recruitment and M2 polarization. It can inhibit T cells and NK cells, and help cancer cells escape immune surveillance and immune defense (86). Other studies have shown that ROS may reduce the effectiveness of PD-1 antibodies (93). Therefore, ROS has a certain inhibitory effect on tumor immunotherapy.

ROS can also act as an intracellular signal in the apoptosis pathway (94). Researchers found that high doses of ROS are a promising cancer treatment strategy. Adriamycin can induce apoptosis by inducing ROS in cells, and HBO can enhance its cytotoxicity. Chunle Zhao found that a large amount of ROS has a killing effect on cancer cells (84). High ROS, as a strong oxide, can induce oxidative stress and activate programmed cell death (95). For example, excessive ROS can inhibit Epidermal Growth Factor Receptor (EGFR)-mediated Phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and block the proliferation of androgen-independent prostate cancer cells (96). ROS can also block the PI3K/AKT/nuclear factor kappa-B (NF-κB) pathway and inhibit the proliferation of non-small cell lung cancer A549 cells (86). ROS can activate p53, which leads to the arrest of the tumor cell cycle (97). ROS can enhance the antigen-presenting ability of dendritic cells, thus triggering the differentiation of monocyte precursors or hematopoietic cells and inducing their maturation (93). In addition, ROS can also reshape or degrade ECM, and serve as a target for anti-tumor therapy. The relationship between ROS production and PD-L1 expression is not clear, but ROS level affects PD-L1 expression in cancer cells (93). It has been proven that ROS combined with PDL-1 blocking can promote the presentation of

tumor antigens to primitive T cells and enhance adaptive anti-tumor immunity (46). Tumor-reactive CTL was isolated from mice treated with anti-PD-L1, and it was found that CTL carried high levels of ROS, which could enhance the activity of PD-1 blockers (93). ROS can also promote intratumoral invasion of CTL and sensitize the tumor to PDL-1-blocking therapy (46). Therefore, ROS can promote immunotherapy to some extent.

The role of ROS in cancer is a double-edged sword. A certain degree of ROS can promote the occurrence and development of cancer, but excessive ROS can induce apoptosis of cancer cells through various mechanisms (84–86, 98). Therefore, HBO can have positive or negative effects on immunotherapy by producing ROS.

The impact of the tumor microenvironment on immunotherapy

Hypoxia and HIF1α can induce immunosuppressive cells contributing to immune tolerance and forming an inhibitory immune microenvironment. Abnormal tumor vascularization can impair blood flow, aggravate hypoxia, and limit the delivery of nutrients and drugs (99). Dense ECM prevents drug penetration into the tumor core, which leads to drug resistance. Therefore, targeting hypoxia and promoting the normalization of tumor blood vessels are helpful to the efficacy of immunotherapy. HBO can regulate the tumor microenvironment and improve cancer immunotherapy by targeting HIF1α, relieving tissue hypoxia, and consuming ECM.

Hyperbaric oxygen and other immunotherapy

Immunotherapy mainly includes ICBs, molecular targeted therapy, adoptive immune cell therapy, cytokine therapy, and tumor vaccine. Antibody therapy is one of the immune therapies. Kun Li et al. found that after teniposide chemotherapy, HBO promoted the recruitment of activated CTL, and the tumor microenvironment changed from a non-inflammatory state to an inflammatory state. HBO combined with teniposide chemotherapy increased the sensitivity of the tumor to PD-1 antibody and improved the therapeutic effect of PD-1 antibody in various tumor models (100). Ustekinumab, as an immunosuppressant, blocks the synthesis of Interleukin (IL)-12 and IL-23 and inhibits the activity of T cells. Lauren E Provini reported for the first time a case of HBO combined with ustekinumab in the treatment of severe suppurative sweat gland inflammation (101). Antivenom is a drug containing specific antibodies. The effect of HBO combined with antivenom was better than that of antivenom alone (102). Rituximab is a monoclonal antibody that targets CD20 cells. A Chinese woman with a severe vasculitis ulcer was treated with rituximab, methotrexate, and HBO, and the ulcer was improved (103). In addition, HBO can increase the curative effect of adalimumab in hidradenitis suppurativa (104). We found that HBO can increase the efficacy of antibody therapy in diseases. Therefore, these applications in other disease states are templates for possible combinations of HBO and cancer antibody therapy.

Practical application of hyperbaric oxygen

The dose of HBO is composed of two independent components, namely hyperoxia and increased barometric pressure. HBO plays an immunomodulatory role depending on oxygen and pressure (105). T Alagoz exposed the mouse tumor model to three 30-minute HBO (100% oxygen pressurized to 2.4 atmospheres) exposures and two 10-minute air interruptions per day. After 5 days of HBO treatment, cisplatin chemotherapy was performed. Finally, T Alagoz's team found that HBO promotes the vascular supply of tumors and helps the delivery of chemotherapy drugs (71). Ingrid Moen divided mice into three groups, one group received intermittent HB0 treatment for three days (1st, 4th, and 7th days), one group received continuous HBO treatment for seven days, and one group served as a control group. HBO treatment was performed by pressurizing 100% oxygen to 2.5 bar for 90 minutes. The final results showed that only after intermittent HBO treatment, the blood vessel density decreased. At the same time, hyperoxia leads to down-regulation of the mitogen-activated protein kinase (MAPK) pathway and inhibits tumor growth (74). Metastatic mouse osteosarcoma cells were treated with HBO (100% oxygen pressurized to 2.5 atmospheres, 5 times a week for 5 weeks) and carboplatin. Yasuomi Kawasoe found that HBO enhanced the chemotherapy effect of carboplatin and significantly inhibited osteosarcoma growth and lung metastasis (106). The mouse H22 subcutaneous tumor model was treated with HBO (pure oxygen pressurized to 2.5 atmospheres) for 1.5 hours, and then the PD-1 antibody was injected intravenously. Xin Liu found that HBO enhanced the immune response of PD-1 antibody and the infiltration of T cells into tumor parenchyma (31). After Xian Wu combined the nano-drug Doxil with HBO (more than 97% oxygen pressurized to 2.5 atmospheres absolute), it was found that collagen deposition decreased and tumor hypoxia eased. Combined therapy synergistically inhibited tumor growth, and the inhibition rate reached 91%. Therefore, the combination of HBO and other nano-drugs may become a safe way to treat tumors (22). Pan Wang used BALB/c-nu mice to inoculate glioblastoma cells into the brains of mice. Mice were injected with temozolomide and exposed to HBO (2.5 atmospheres of pure oxygen) for 90 minutes. The results showed that HBO treatment alone might promote tumor growth. The tumor volume of mice in HBO combined with the temozolomide group decreased and the survival time was prolonged. HBO combined with temozolomide can inhibit HIF1α and HIF2α expression and promote chemical sensitization (66). Xiaoxian Wang used HBO (pure oxygen pressurized to 2.5 atmospheres absolute) in combination with Abraxane, and gemcitabine. HBO inhibits CAFs, normalizes tumor vessels, and enhances the anti-tumor activity of drugs (64). Shao-Yuan Chen exposed metastatic cells to HBO (98% oxygen, 2.5 atmospheres absolute). As a result, HBO improved tumor vascular hypoxia and targeted tumor apoptosis-related genes (54). After

HBO (> 97% oxygen, pressure 2bar) treatment, tumor vessel density decreased and tumor cell apoptosis increased (75). Yong-Gang Wang established a mouse glioma model. After HBO (100% oxygen, 2.5 atmospheres) treatment, the ROS level was evaluated by flow cytometry. They found that HBO reduced ROS levels in brain cells and raised ROS levels in the thymus. Finally, it inhibits T-cell maturation and promotes the growth of malignant tumors (90). Chunxia Chen found that ROS and lipid ROS levels in HT22 cells and PC12 cells decreased after HBO (pure oxygen, 0.25 MPa) treatment, thus protecting cells from oxygen-glucosedeprivation (107). However, Qin Hu et al. found that delaying HBO (2.5 atmospheres absolute) significantly increased ROS level, which may improve the long-term rehabilitation of stroke patients through the ROS/HIF-1 α/β-catenin pathway (108).

In short, in the process of practical application of HBO, different doses and exposure modes have different effects on tumor growth (Table 1). HBO may play a dual role in tumor angiogenesis and ROS generation.

Discussion

Immunotherapy has achieved great success since its debut. It has shown strong anti-tumor activity in the treatment of solid tumors such as melanoma (109), non-small cell lung cancer (110), renal cell cancer (111), and prostate cancer (112), which has changed the pattern of tumor treatment to a certain extent. However, clinical drug resistance limits its development (6). In recent years, there have been many studies on drug resistance in immunotherapy. Esther Redin found that dasatinib increased the antitumor activity of anti-PD-1 by inhibiting the transformation of Treg cells (113). Guohao Wang believes that nano units can

TABLE 1 Practical application of hyperbaric oxygen.

Author	Oxygen concentration	barometric pressure	HBO exposure time per day/minutes	Days	Function	Reference
T Alagoz	100%	2.4atm	90 (30 minutes HBO+10 minutes air+30 minutes HBO +10 minutes air+30 minutes HBO)	5 days in a row	Promote tumor angiogenesis	(71)
Ingrid Moen	100%	2.5bar	90	Day 1, 4, 7	Down-regulate the MAPK pathway and reduce the density of vascular	(74)
Yasuomi Kawasoe	100%	2.5atm	60	5 times a week for 5 weeks	Enhance the effect of chemotherapy	(106)
Xin Liu	100%	2.5atm	90	Day 1, 3, 5	Enhance the immune response of PD-1 antibody to tumor	(31)
Xian Wu	>97%	2.5ata	120	3 days in a row	Relieve tumor hypoxia	(22)
Pan Wang	100%	2.5atm	90	15 days in a row	Inhibit the expression of HIF1 α and HIF2 α	(66)
Xiaoxian Wang	100%	2.5ata	90	Day 1, 2, 3, 4, 7, 10	Inhibit Cancer-Associated Fibroblasts	(64)
Shao-Yuan Chen	98%	2.5ata	90	14 days in a row	Improve tumor angiogenesis	(54)
Linda Elin Birkhaug Stuhr	>97%	2bar	90	Day 1, 4, 7	Induce apoptosis of tumor cells	(75)
Yong-Gang Wang	100%	2.5atm	60	10 days in a row	Inhibit T cell maturation	(90)
Chunxia Chen	100%	2.46atm	60	Day 1	Reduce ROS in cells and lipids and inhibit iron death	(107)
Qin Hu	–	2.5ata	90	One cycle is 7 consecutive days, with a rest of 5 days. Three cycles	Promote neural function recovery through ROS/HIF-1 α/β-catenin pathway	(108)

atm, atmospheres; ata, atmospheres absolute; MAPK, Mitogen-activated protein kinase.

enhance the response to PD-L1 checkpoint blocking (114). We searched for targets and therapeutic strategies for immunotherapy resistance at gene and molecular levels, which suggested the importance of the tumor microenvironment for immunotherapy. HBO therapy has a long history. Recently, the combination of HBO with radiotherapy, chemotherapy, and photodynamic therapy has shown good therapeutic effects (115). Therefore, we may also consider combining HBO with cancer treatment to explore its impact on cancer treatment.

Most cancer patients will have an imbalance of immune system function. Considering the influence of HBO on the immune system and its potential therapeutic effect in autoimmune diseases, the combination of HBO and immunotherapy is a promising therapeutic strategy. HBO improves tumor hypoxia by down-regulating HIF1α (64). Targeting HIF1α in immunotherapy is a relatively new concept and its rationale has been well-documented by others (18, 19, 62). HIF1α is usually inactivated in normal tissues, but it is usually stable in tumor cells, regardless of oxygen tension. Targeting HIF1α has been shown to isolate immunotherapeutic effects and reduce the incidence of immune-related adverse events in preclinical models (116). HBO normalizes the vascular composition around the tumor. HBO depletes ECM collagen fibrils, collagen I, and fibronectin (63). HBO can regulate the tumor microenvironment by increasing the proportion of MI and M2 phenotype macrophages and effector memory T cells. Finally, HBO has also been found to promote the infiltration of PD-1 antibodies and T cells into solid tumors (31). But HBO therapy has not been shown clinically to affect cancer in any significant way by itself, which strongly suggests that it must be used in combination with immunotherapy. In addition, HBO enhances the therapeutic effect of antibodies in non-cancer diseases. Antibody therapy is a type of immunotherapy. Therefore, We can consider combining HBO with immunotherapy for cancer.

But HBO can also produce ROS while regulating the tumor microenvironment. Different levels of ROS in cancer treatment are a double-edged sword. The amount of ROS produced *in vivo* by HBO therapy lacks specific metrics to determine. Therefore, The suppressive effect of HBO therapy on immunotherapy also needs to be considered.

Many studies have been conducted today to overcome tumor hypoxia, such as using HBO therapy, oxygen delivery by nanocarriers (117–119), normobaric hyperoxia (62, 120), vascular normalization to enhance blood perfusion and oxygenation (121), and reduction of cellular oxygen consumption (122). These approaches have been shown to activate CTL and enhance ICBs through antibody-mediated immunotherapy. However, most of these studies exist in preclinical models and there is still a long way to go before they can be truly applied in clinical practice. For example, Normobaric hyperoxia, a relatively well-established clinical oxygenation strategy, has been found to enhance anti-tumor activity by suppressing tumor-reactive immune cells. However, HBO is not normobaric hyperoxia. HBO increases the air pressure at the same time as increasing the oxygen concentration. Stress genes are very important, and HBO can inhibit pro-inflammatory genes and affect immune response. Therefore, HBO plays an immunomodulatory role through hyperoxia and high pressure (62).

While HBO therapy is expected to overcome hypoxia by increasing the oxygen supply to the tumor tissue, its beneficial effects are varied. HBO therapy varies depending on the type of tumor, the size of the lesion, and the clinical status of the patient. Therefore, the application time, duration, and dose of HBO are very important (49). We found that in the practical application of HBO, the commonly used dose is 100% oxygen and 2.5 atm. HBO treatment for 90 minutes every day for 3-7 days may inhibit tumor growth and promote chemotherapy and immunotherapy of cancer. However, the best dose and exposure mode of HBO to promote cancer immunotherapy need further study and verification.

Malignant tumor has been considered a contraindication of HBO therapy in the past, so the application of HBO in cancer is relatively rare. Today, most studies combine HBO with radiotherapy, photodynamic therapy (11), and nano-drugs (12, 63). The combination of HBO and immunotherapy is relatively rare. We found that HBO can resist the drug resistance of immune checkpoints to a certain extent and promote the immunotherapy of cancer. This paper summarizes how HBO therapy affects cancer immunotherapy by regulating the tumor microenvironment, which provides a breakthrough point for immunotherapy and may enlighten the future direction of immunotherapy.

Author contributions

PW, X-YW, and C-FM collected the related paper and finished the manuscript and figures. YF and D-DG gave constructive guidance and made critical revisions. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

ICBs	Immune checkpoint blockers
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death ligand 1
HBO	Hyperbaric oxygen
ECM	Extracellular matrix
HIF1α	Hypoxia-inducible factor 1α
MDSC	Myeloid-derived suppressor cells
VEGF	Vascular endothelial growth factor
PDGF	Platelet-derived growth factor
CTGF	Connective tissue growth factor
CTL	Cytotoxic T lymphocyte
EMT	Epithelial-mesenchymal transition
HCC	Hepatocellular carcinoma cell
LncRNA	Long non-coding RNA
Treg	Regulatory T cells
NK	Natural killer
CAFs	Cancer-associated fibroblasts
CLL	Chronic lymphocytic leukemia
ROS	Reactive oxygen species
M1	Macrophages1
M2	Macrophages2
EGFR	Epidermal Growth Factor Receptor
PI3K	Phosphatidylinositol 3-kinase
NF-κB	Nuclear factor kappa-B
IL	Interleukin
MAPK	Mitogen-activated protein kinase
DC	Dendritic cells
TCR	T cell receptor
MHC	Major histocompatibility complex
CD	Cluster of differentiation
VHL	Von Hippel Lindau
atm	atmospheres
ata	atmospheres absolute

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Hyperbaric oxygen therapy as a complementary treatment in neuroblastoma — a narrative review

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Neuroblastoma is the most frequently diagnosed cancer during the first year of life. This neoplasm originates from neural crest cells derived from the sympathetic nervous system, adrenal medulla, or paraspinal ganglia. The clinical presentation can vary from an asymptomatic mass to symptoms resulting from local invasion and/or spread of distant disease spread. The natural history of neuroblastoma is highly variable, ranging from relatively indolent biological behavior to a high-risk clinical phenotype with a dismal prognosis. Age, stage, and biological features are important prognostic risk stratification and treatment assignment prognostic factors. The multimodal therapy approach includes myeloablative chemotherapy, radiotherapy, immunotherapy, and aggressive surgical resection. Hyperbaric oxygen therapy (HBOT) has been proposed as a complementary measure to overcome tumor hypoxia, which is considered one of the hallmarks of this cancer treatment resistance. This article aims to review the relevant literature on the neuroblastoma pathophysiology, clinical presentation, and different biological and genetic profiles, and to discuss its management, focusing on HBOT.

KEYWORDS

neuroblastoma, treatment, therapy, iodine radioisotopes, iodine-131, radiopharmaceuticals, hyperbaric oxygenation, hyperbaric oxygen therapy

1 Background

Although neuroblastoma is extremely rare in adults, it is the most frequently diagnosed cancer during the first year of life. Corresponding to more than 7% of neoplasms in patients younger than 15 years, it accounts for about 15% of pediatric cancer deaths. The age of presentation is between 18 and 22 months, with most cases diagnosed before 5 years of age. Despite being more frequent in Caucasian children, race does not seem to impact the prognosis (1–3).

The etiological factors are unknown due to the low prevalence of this disease. The potential role of maternal exposures during pregnancy, namely tobacco, alcohol, drugs and medication, chronic or infectious diseases, and vitamin supplements, has not yet been unequivocally established. While most neuroblastomas occur sporadically, about 1% of cases are hereditary by an autosomal dominant pattern with incomplete penetrance. These have distinctive characteristics: early appearance, multiple primary tumors, and good prognosis. In 5% of cases, there is an association with congenital diseases, namely von Recklinghausen's disease, neurofibromatosis type 1, Hirschsprung's disease, and other neurocristopathies, suggesting a common genetic background (1–3).

This neoplasm originates from neural crest cells derived from the sympathetic nervous system, adrenal medulla, or paraspinal ganglia. The clinical presentation can vary from an asymptomatic mass to symptoms resulting from local invasion and/or distant disease spread. The natural history of neuroblastoma is highly variable, ranging from relatively indolent biological behavior to a high-risk clinical phenotype with a dismal prognosis (1–3).

Age, stage, and biological features found in tumor cells are important prognostic factors for risk stratification and treatment assignment. The multimodal therapy approach includes myeloablative chemotherapy (CTX), radiotherapy (RT), immunotherapy (IMT), and aggressive surgical resection (1–3).

Hyperbaric oxygen therapy (HBOT) has been proposed as a complementary measure to overcome tumor hypoxia, which is considered one of the hallmarks of cancer treatment resistance. Even when isolated, HBOT appears to interfere with tumor growth, metastases, angiogenesis, and anticancer gene expression. Additionally, combining HBOT with specific treatments enhances the production of reactive oxygen species (ROS), inducing apoptosis (4). In this setting, HBOT has been clinically successfully tested with meta-iodobenzylguanidine (^{131}I-MIBG) in recurrent neuroblastoma stage IV by combining various biochemical and cellular mechanisms that lead to tumor cell death. Currently, HBOT is considered by the European Committee for Hyperbaric Medicine (ECHM) as a modality of treatment for recurrent neuroblastoma stage IV (degree of recommendation II/level of evidence C) (5).

This article aims to review the relevant literature on the neuroblastoma pathophysiology, clinical presentation, and different biological and genetic profiles, and to discuss its management, focusing on HBOT.

2 Pathophysiology

The term neuroblastoma refers to a group of neoplasms of common origin, recognized by James Homer Wright as neuroblastic tumors. The spectrum of neuroblastic tumors is classically classified into three groups with different biological behaviors, reflecting the increasing maturation of sympathetic nervous tissues: ganglioneuromas (benign behavior), ganglioneuroblastomas (intermediate behavior) and neuroblastomas (malignant behavior). More recently, the International Neuroblastoma Pathology Committee (INPC) re-divided it into four categories: neuroblastoma (schwannian stroma-poor tumors), intermixed ganglioneuroblastoma (schwannian stroma-rich tumors), nodular ganglioneuroblastoma, and ganglioneuroma (schwannian stroma-dominant tumors). Each category includes several subtypes according to the degree of cell differentiation (1–3).

Neuroblastoma is an embryonal neuroepithelial malignancy of the sympathetic nervous system arising from neuroblasts (pluripotent sympathetic cells). Under normal conditions, neural crest cell precursors invaginate, migrate along the neuroaxis, and populate the sympathetic ganglia, adrenal medulla, and other sites. However, migration, maturation, or differentiation defects can lead to neuroblastoma formation, typically composed of immature small round blue cells. Thus, the origin and migration pattern of neuroblasts during fetal development explains the anatomic distribution pattern along the peripheral sympathetic nervous system: abdominal cavity (40% adrenal, 25% paraspinal ganglia), chest (15%), pelvis (5%), cervical (5%), miscellaneous (12%), and even occult (1%). This cancer metastasizes via lymphatic and hematogenous dissemination, most commonly in the lymph nodes, bone marrow, cortical bone, and liver (1–3).

Several chromosomal and molecular markers have been extensively investigated in the last decades. Still, data is scarce concerning the genes involved in interrupting normal neuroblast differentiation, their malignant transformation, and neoplastic progression. Concurrently, these biological markers were evaluated to determine their value in predicting prognosis, with some being incorporated into the strategies used for risk stratification (1–3).

In 1983, a gene frequently amplified in cells was identified: the proto-oncogene MYCN (located on the short arm of chromosome 2). The MYCN gene is a member of the MYC family of transcription factors and encodes a protein with a basic helix-loop-helix protein 37, also known as N-myc proto-oncogene protein. In about 20% to 25% of neuroblastomas, MYCN amplification is observed, with consequent activation. Deregulated gene expression was tested in mice, which eventually developed neuroblastomas, proving their involvement in oncogenesis. Patients whose tumors have MYCN amplification tend to have rapid tumor progression and poor prognosis, even with other favorable factors, such as low-stage disease or IVS disease (1–3).

Another interesting finding is an increase in the expression of targeted genes. Furthermore, anaplastic lymphoma kinase (ALK) is highly expressed, mainly in hereditary, and in 5-15% of cases from somatic origin. Essentially, single-base mutations occur in the kinase domain, leading to constitutive activation of the kinase domain and, subsequently, contributing to a premalignant lesion (2). Also, the activation of the ALK oncogene can increase cell proliferation and survival, making it a great candidate for specific biological targeted therapy (3).

The association with other pathologies, such as Hirschsprung's disease and/or central hypoventilation syndrome, has also

demonstrated that loss-of-function mutation may happen in the homeobox gene PHOX2B. Consequently, if the patient has a positive family history of and/or the aforementioned diseases, ALK and PHOX2B mutations should be genetically screened (2).

Moreover, the Children's Oncology Group (COG) study has concluded that few alleles might contain single-nucleotide-polymorphism mutations that are more present in children than in healthy controls, essentially the ones in the gene FLJ22536 at chromosome 6 long arm position 22.3 (6p22.3), gene BARD1 (BRCA1-associated Ring Domain 1) at 2q35, as well as at 1q21 (2).

The tallying of the genetic mutations involved in the onset and development of neuroblastoma can be divided into two main categories: 1) hyperdiploidy, which contains modifications at a whole chromosome level (correlated with better outcomes); 2) segmental chromosomal changes (amplification of M-YCN, loss-of-heterozygosity in 11q, 1p, gain of function at 17q and activation of ALK) (related to worse prognosis) (1, 2).

3 Clinical presentation

As the sympathoadrenal lineage of the neural crest remains the origin, this tumor may arise anywhere in the sympathetic nervous system. Hence, the primary tumor site is within the abdominal cavity, followed by the adrenal medulla. Due to its inherent heterogeneity, the clinical presentation is highly variable and reliant on tumor location.

An infant with an abdominal mass without pain is usually the primary setting of this disease. Most of the symptoms will result from the mass effect of the tumor on surrounding structures such as 1) airway, originating dyspnea; 2) great vessels, leading to ischemia and, subsequently, necrosis; 3) spinal cord, present in 5% of patients, and causing neurological symptoms like paraesthesia/anesthesia, motor muscle weakness or significant pain. Furthermore, systemic non-specific symptoms, like fever, weight loss, and asthenia, may arise (3).

Moreover, metastatic disease is another reason for symptomatic manifestations. The principal metastatic sites include cortical bone, non-contiguous lymph nodes, liver, and bone marrow. This neoplasm frequently spreads to the ostial part of the ocular orbit, originating proptosis and/or periorbital ecchymosis, known as 'Racoon eyes' (1). Skin can also be affected by metastatic subcutaneous nodules (3). After bone marrow invasion, patients may present arterial hypertension due to a renin-dependent mechanism, irritability, and intense bone pain (1).

Finally, the paraneoplastic syndrome may also be responsible for symptoms, being more associated with localized disease, thus with a better prognosis. If the mass has a cervical location, Horner syndrome might arise. The two principal paraneoplastic syndromes include 1) increased secretion of vasoactive intestinal peptide, manifesting as refractory aqueous diarrhea; 2) opsoclonus-myoclonus, manifesting as a triad of symptoms that consist of ataxia, irregular muscle movements, and nystagmus (present in 2-4% of patients) (1, 3).

4 Diagnosis

The first necessary approach is primary tumor assessment through cross-sectional image exams, such as computed tomography (CT) or magnetic resonance imaging (MRI). Choosing one method over another is based on the inferred location: CT is indicated in the mediastinal, abdominal, or pelvic area. The MRI should be used for the spinal cord (1, 3).

As the staging is the subsequent phase, CT and/or MRI could further evaluate local and distant invasion. The ^{131}I-MIBG scintigraphy is also important in detecting bone or soft tissue metastases, being the preferred method due to its enhanced specificity and sensibility. If doubt remains, single photon emission CT (SPECT/CT), 18-fluorodeoxyglucose (FDG) PET/CT, and technetium 99m bone scintigraphy may be indicated (1).

Since bone marrow involvement is relatively frequent, aspirates and, subsequently, biopsies should be performed, after which it is essential to apply immunocytochemical and PCR techniques for the detection of cells and specific transcripts such as PgP9.5, GD2 synthase, and tyrosine hydroxylase (3).

Finally, a biochemical analysis helps to assess the prognosis, specifically the vanillylmandelic acid/homovanillic acid ratio, lactate dehydrogenase, and serum ferritin (3).

5 Treatment

Since pathophysiology is not yet fully understood, treatment is highly heterogeneous and relies on the prognostic risk based on tumor resectability, biological, genetic, clinical, and histological features. Overall, it is possible to understand that non-high-risk tumors tend to have reduced-to-non-treatment, contrarily to high-risk neoplasms (1–3).

Patients under 6 months of age, adrenal solid tumors with less than 3.1 cm, or adrenal cystic tumors with less than 5.0 cm only need expectant observation. In patients with stage I from INSS (International Staging System) (6), consisting of "localized tumor with complete gross excision with or without microscopic residual disease," only surgical resection is required, independently of biological features (3).

For patients with stages IIA and IIB from INSS, who have localized tumor that is not fully resectable but has favorable characteristics, surgery is essential, and CTX is selectively applied if the location is life- or organ-threatening or if the disease is progressive or recurrent (3).

Finally, patients with stage III from INSS, which comprises: "unresectable unilateral tumors across the midline," and children less than 18 months of age, with stage IV from INSS, which is "any primary tumor with dissemination to distant lymph nodes, bone, bone marrow or other organs" are classified as intermediate risk and need both surgery and CTX (3).

A subset of stage IV is still considered a non-high risk when the patient is less than 18 months old, with a small-sized tumor, metastases restricted to bone marrow (< 10% involvement), liver and skin, and favorable histology and biology (such as the presence of hyperdiploidy and single-copy MYCN). In this subgroup, minimal support therapy is needed, with CTX and surgery only performed in patients with disseminated intravascular coagulation or respiratory distress induced by significant abdominal involvement (3).

The primary challenge in neuroblastoma treatment remains high-risk tumors, namely stage IV from INSS disease with unfavorable characteristics, especially recurrent or relapsed pathology. The main issue regarding this kind of tumor presentation is the occurrence of extensive hypoxic areas that can remarkably increase the resistance capacity to CTX and RT. The high-risk neuroblastoma treatment regimen can be divided into three sections: 1) remission induction, where the goal is to eliminate the tumors at gross; 2) remission consolidation, to avoid tumor regrowth; 3) maintenance, to eradicate minimal residual disease and prevent relapse (2).

In the first phase, the main CTX regimen, introduced by the Memorial Sloan-Kettering Cancer Center, is comprised of 5 cycles of intensive induction treatment (vincristine, doxorubicin, and cyclophosphamide interspersed with cisplatin and etoposide) with a collection of peripheral blood stem cell at the end of 3rd cycle. Subsequently, the patient's response is evaluated, which may constitute a prognostic biomarker (2, 3).

For the consolidation regimen, platin salt and etoposide are maintained, with additional cyclophosphamide pursued by a combination of thiotepa and cyclophosphamide and local RT. Other therapeutic regimens include single bone marrow transplantation or cyclophosphamide plus thiotepa followed by cyclophosphamide, etoposide, melphalan, or alternatively, busulfan combined with melphalan (3).

At last, aiming to eradicate residual disease and prevent relapse, some targeted therapies are being studied. One of the first approaches for refractory or relapsed high-risk neuroblastoma patients was ^{131}I-MIBG therapy. It uses ^{131}I-MIBG-targeted radionuclides to achieve selective radioactive treatment exclusively in ^{131}I-MIBG-producing cells, essentially tumor cells (3). Recently, a meta-analysis with 26 clinical trials and 883 neuroblastoma patients reported a pooled rate of objective response of 39% and 28% for ^{131}I-MIBG monotherapy and in combination with other therapies, respectively. The pooled occurrence rates of thrombocytopenia and neutropenia in ^{131}I-MIBG monotherapy studies were 53% and 58%. In a combination regimen, the pooled occurrence rates of thrombocytopenia and neutropenia were 79% and 78% (7). The primary underlying mechanism relies on the augmented expression of noradrenaline transporters, which enhances ^{131}I-MIBG intake into tumor cells (7–13).

Furthermore, alternative therapies include retinoids since their capacity to increase the terminal differentiation of neuroblastoma cells has previously been demonstrated. Moreover, as this tumor develops hypoxic areas, angiogenesis inhibitors might decrease neuroblastoma aggressiveness and, consequently, prevent cancer relapse (1).

Another relevant target is disialoganglioside GD2, expressed on the surface of this tumor's cells. An antibody against GD2 has already been developed in animal models and has shown promising results in eliminating metastatic disease. The chimeric anti-GD2 monoclonal antibody ch 14.18, in combination with granulocyte-macrophage-colony stimulating factor and interleukin-2, revealed an exciting amelioration of 2-year EFS (2).

Moreover, as neuroblastoma is highly heterogeneous and, as a consequence, multiple genetic biomarkers have already been identified (e.g., MYCN, ALK), several research groups are developing antibodies against specific genetic biomarkers to counteract the aggressive replication of neuroblastoma cells, as well as modifying the tumor microenvironment (TME), which is intrinsically associated with tumor growth and immune system evasion (11–13).

Countless therapeutic approaches are being investigated to evade neuroblastoma resistance and relapse. One of the most promising treatments may be HBOT.

6 Hyperbaric oxygen therapy

The word "baric" and its prefix "hyper" define the purpose of HBOT, which consists of breathing 100% of O_2 under hyperbaric conditions, frequently ranging between 2.0 and 2.8 atmospheres absolute (ATA) (14). Its main effect is exerted by the elevation of the inspired gas partial pressure, together with an increase in the fraction of inspired O_2 (15), resulting in the enhanced amount of O_2 dissolved in plasma and an increase in the O_2 delivered to tissues, independently of hemoglobin (4, 16–18).

Hemoglobin is mainly responsible for carrying O_2 in the blood, with O_2 saturations of ~95% under normal atmospheric pressure, while 0.32% is dissolved in the plasma (14). In these conditions, the total O_2 content in the blood is ~21 ml O_2/dl, with tissues consuming an average of 5 to 6 ml O_2/dl blood. Because hemoglobin becomes fully saturated with 100% O_2 at sea level (normobaric pressure), the only way we have to change the blood O_2 content is to raise its amount dissolved in the plasma (19). Henry's law states that the amount of dissolved gas in a liquid is directly proportional to the partial pressure of the gas above that liquid. Therefore, breathing 100% O_2 at 3 ATA means we will have partial pressures higher than 2000 mmHg. Taking into account the solubility factor for O_2 (0.0024 mL O_2/dl of blood per mmHg), we can reach at least 4.8 ml O_2/dl blood dissolved in plasma and a total O_2 content of 24.8 ml O_2/dl blood, a net increase of 4.56 ml O_2/dl blood or 22.5%, thus enhanced the delivery of that gas to tissues (19).

The way that HBOT affects the body depends on direct and indirect physiologic effects and defines its therapeutic use and main clinical applications. The primary effect is the correction of hypoxia as a direct consequence of ischemia or impairment of O_2 transport to tissues. Hypoxia is a major factor in developing several pathological conditions and is a hallmark in cancer progression today.

7 Application of hyperbaric oxygen therapy in neuroblastoma

7.1 Tumor hypoxia

Like many other solid tumors, neuroblastoma is mainly driven by genetic alterations that substantially define its progression and malignancy. Nonetheless, recent data revealed that the interplay mechanisms of the TME play a significant role in the progression and metastases of this neoplasm (20).

A common physiologic condition, which can equally be a cause and a driving force to these alterations, is found: hypoxia. This refers to a deficit in O_2 delivery to organs, tissues, and cells, meaning an imbalance in supply and demand to fulfill the so-called baseline functions necessary for homeostasis (19). That imbalance can be transitory, acute, or chronic depending on underlying causes and physiological conditions. Despite the differences in O_2 tensions and demands of individual tissues, on average, they consume 5 to 6 ml O_2/dl blood at rest, necessary to maintain regular metabolic functions. Therefore, we can properly define hypoxia as a condition where tissues fail to receive the usual amount of O_2, which, if not corrected, leads to several modifications at a cellular level, organ dysfunction, and, ultimately, death (19). The body has several mechanisms to adjust and maintain adequate tissue oxygenation, such as ventilatory rate, cardiac output, stroke volume, dilatation and/or constriction of the capillary bed. Nonetheless, the cellular responses play a significant role, where hypoxia begins to determine the appearance and persistence of the disease (19). At this cellular level, O_2 is a critical component in energy production, used as the final receptor in the electron transport chain to form adenosine triphosphate. Less O_2 in the cell means an energy deficit and cell death occurs if it persists too long (19). Additionally, some studies report exposure to hypoxia with an increase in oxidative stress, with low levels of O_2 favoring the production of ROS and reactive nitrogen species. Both these components have essential roles in hypoxic signaling pathways and their apparent implications in several pathological conditions, particularly cancer development (21).

The concern with tumor hypoxia began in the twentieth century with the work of Mottram JC, who, in 1936 was already concerned with the conditions in which tumor cells could survive, correlating resistance to RT with a reduced O_2 supply in tumors (22). With scientific progress, it was possible to identify and characterize tumor hypoxia as one of the main features of cancer progression and malignancy (23). The decreased O_2 levels harm normal and cancer cells, but the latter develop several adaptive strategies to survive and proliferate under hypoxic conditions (24). Höckel M and Vaupel P, in their review of biological and molecular aspects of tumor hypoxia, state that this phenomenon occurs due to poor microcirculation and diffusion conditions (25). In fact, we know today that these structural abnormalities in tumor vasculature disrupt blood flow and widen the distance of O_2 diffusion from blood vessels, leading to acute and chronic hypoxia in surrounding tissues (26). Contrarily to healthy tissues, tumors are unable to regulate the diminishing O_2 levels, leading to the development of hypoxia (26).

Despite the type of identified hypoxia, acute (cycling hypoxia) or chronic, both act in tumor cells and the so-called TME, affecting cancer progression, angiogenesis, metastases, and resistance to therapy. Several of the hypoxia-induced pathways are directly correlated with the well-described hallmarks of cancer, namely inducing angiogenesis, resisting cell death, enabling replicative immortality, activating invasion and metastases, evading growth suppressors, and sustaining proliferative signaling (27). If we look closely at one of the principal pathways, which includes the hypoxia-inducible transcription factor 1 (HIF-1), the importance of this physiological condition in cancer biology becomes apparent. In the absence of O_2, HIF-1 promotes several changes that will affect tumor cells in various ways (28): regulation of genes and control of malignant and metastatic phenotype of cancer cells by enhancing cell survivor through growth factors and inhibition of apoptosis; promotion of tumor growth by angiogenesis and neovascularization, mediated by VEGF and bone marrow-derived endothelial progenitor cells; increase in metastatic activity, particularly via epithelial-to-mesenchymal transition, a process that alters the cell phenotype to acquire mobility and migration capacities; resistance to drug treatments by cellular processes of quiescence, inhibition of apoptosis, controlling autophagy, as well as by the lack of O_2 required for the CTX cytotoxicity. In addition to the genetic and cellular modifications, hypoxia also affects TME, inducing metabolic and molecular changes in endothelial cells, regulation of inflammatory mediators and growth factors, as well as affecting stromal cells, immune cells, and non-cellular components like extracellular matrix, cytokines and other mediators (27). Considering all those changes and interactions in the biology of cancer promoted by hypoxia, it seems logical that one of the therapeutic targets of cancer, specifically in treating neuroblastoma, should be the correction and overcoming of tumor hypoxia.

Focusing on the HIF family, the primary mechanism of this family consists in the dimerization of the subunits – alpha, which is O_2-dependent (HIF-1α, HIF-2α, and HIF-3α), and beta, which is O_2-independent. Under normoxic conditions, the alpha subunits of HIF are usually targeted for ubiquitination by the hydroxylation of prolyl particles and, later, are connected with von Hippel Lindau (VHL) proteins, leading to end-stage HIF alpha subunit degradation. However, under hypoxic conditions, the HIF subunits follow a different pathway. HIF-1α proteins respond acutely to low levels of O_2 (around 1-2%), while HIF-2 α is only induced in extended periods of hypoxia. Additionally, it appears that the downstream signaling of HIF-1α, HIF-2α, and HIF-3α is similar amongst this family (29, 30).

Interestingly, neuroblastoma cells seem to express enhanced levels of HIF-1α and HIF-2α genetic material, with discrepant meaning. When the expression of HIF-1α is superior to HIF-2α, the patients show a more favorable prognosis with a less aggressive tumor phenotype. However, when the opposite occurs, the tumor has a higher probability of being found in higher stages of development with a subsequent worse prognosis associated (30, 31). Furthermore, it was noticed that clinical neuroblastoma samples seem to present increased concentrations of tumor cells with HIF-2α near blood vessels. This means that HIF-2α can be a biomarker for specific populations of neuroblastoma stem-cell-like and/or neural crest cells. Still, it may also lead to increased

production of VEGF and other cytokines responsible for neovascularization, consequently leading to more aggressive phenotypes (30–32).

Therefore, HIF proteins, especially HIF-2α, are presented as potential targeted therapies. Thus, to overcome these hypoxia-induced proteins, there is a need to change the hypoxic TME. One way to accomplish that could be through HBOT.

7.2 Hyperbaric oxygen therapy and neuroblastoma

Nonetheless, several doubts arise about whether HBOT would promote the malignant growth of tumors. Suppose HBOT is an adjunctive therapy in wound healing, promoting the proliferation of fibroblasts, epithelial cells, and blood vessels. Could it not have the same effect on malignant tumors (33)?

However, a recent review by Feldmeier J et al. revealed that HBOT not only had no enhancing effect on tumor growth or promoting *de novo* cancer, but it may also have a cancer inhibitory effect as a radiosensitizer (33). Concerns about the possibility of HBOT promoting tumor angiogenesis, similar to the same wound healing process, do not consider the different natures of tumor vasculature and several other pathways for cancer growth. The authors conclude that HBOT administration should not be withheld in patients for whom its benefit is proven, such as delayed radiation-induced injuries, due to concerns that this treatment may cause tumor recurrence or metastases. Additionally, a study about the effect of HBOT on human oral cancer cells, using apoptosis and proliferating cell nuclear antigen to evaluate tumor progression, showed no evidence for the growth or proliferation of cancer cells after HBOT (34).

The HBOT has been studied as a complementary treatment to enhance the effects of RT and CTX, with promising results. As Alpuim Costa D et al. pointed out, "the presence or absence of molecular O_2 dramatically influences the biological effect of radiation exposure" (4). Already by 1953, Gray LH et al. showed that the sensitivity of tumor cells to X-rays was about three times greater when irradiated in a well-oxygenated environment when compared to low concentrations of O_2; they also concluded that the effectiveness of RT was likely to increase if the patients were breathing O_2 at the time of irradiation (35). The same authors proved that the O_2 concentration influences the radiosensitivity of tumor cells at the time of irradiation (35). In the absence of O_2, the radiation dose needed to achieve the same biological effect is three times higher (36–38). Ionizing radiation damages DNA directly and indirectly. Indirect damage occurs through radiation formation of ROS, which is O_2 dependent. When water molecules are exposed to ionizing radiation, they undergo radiolysis, forming hydroxyl and hydrogen radicals. Hydrogen radicals react with O_2, producing perhydroxyl radicals, causing irreversible damage to DNA. The TME with high O_2 levels leads to increased production of ROS and, consequently, to a higher effect of radiation. In 2007, Overgaard found that among several modified methods of hypoxemia to overcome tumor hypoxia, HBOT had the most pronounced effect (39). The efficacy of HBOT in reducing tumor radioresistance has been demonstrated in experimental studies and clinical trials (40, 41). Moreover, HBOT at 2 ATA increased the effectiveness of RT in head and neck tumors and achieved promising results in the local control of high-grade gliomas (41, 42). Additionally, in a pilot study by Dowling S et al. HBOT was safely used with a perfluorocarbon-based solution (Fluosol DA 20%) combined with RT (43).

From another integrative perspective, HBOT and the ketogenic diet (KD) were explored as non-toxic therapies that exploit overlapping metabolic deficiencies of cancer. Cancer cells often exhibit a distinctive metabolic preference for glucose, a phenomenon known as the Warburg Effect or aerobic glycolysis. Cancer cells heavily rely on glycolysis to convert glucose into energy, unlike normal cells, even when oxygen is readily available. This distinct metabolic behavior leads to cancer cells having a significantly higher glucose uptake than normal cells. The KD is a low-carbohydrate, high-fat diet that decreases blood glucose and elevates blood ketones, which can be used as an alternative source. Abnormal tumor vasculature creates hypoxic regions that promote cancer progression and further increase the glycolytic dependency of cancers. Some animal and human experiments have explored the KD's potential to hinder cancer cells' survival by restricting their access to glucose. The rationale for this emerging combination is that while the KD limits glucose availability for cancer cells, HBOT increases oxygen availability. This strategy could create a hostile environment for cancer cells. Promising preclinical research supports this idea, but further rigorous clinical studies are essential to determine the effectiveness and safety of this approach in cancer treatment (4, 44–47).

7.2.1 Hyperbaric oxygen therapy combined with ^{131}I-meta-iodobenzylguanidine

The indication for ^{131}I-MIBG therapy in neuroblastoma is established in the *European Association of Nuclear Medicine procedure Guidelines* (48), being indicated for stage II or IV neuroblastoma in lesions with adequate uptake and retention of ^{131}I-MIBG in pretherapy ^{123}I/^{131}I-MIBG scintigraphy. If ^{131}I-MIBG does not accumulate in primary neuroblastoma lesions, it should not be used as therapy.

The ^{131}I-MIBG is an aralkylguanidine formed by combining the benzyl group of bretylium and guanidine group of guanethidine. This drug is structurally similar to the neurotransmitter noradrenaline. Its uptake by tumor cells is derived from the primitive neural pathway through an active noradrenaline transport system (uptake-1) or passive diffusion. After absorption by cells, it is stored in the cytoplasm and neurosecretory granules, not being metabolized by enzymes. The MIBG labeled with ^{131}I is used as a radiotherapeutic agent in neuroectodermal tumors. Decomposition of decaying ^{131}I radionuclide results in the emission of ionizing radiation, which has an average tissue range of about 0.5 mm. It was first used in treating neuroblastoma in 1986, and since then, several clinical trials have been developed, such as using ^{131}I-MIBG combined with HBOT.

In 1995, Voûte PA et al. compared two groups of patients with recurrent stage IV neuroblastoma after CTX treatment (49). One group was treated with ^{131}I-MIBG (n= 36), and another with ^{131}I-MIBG combined with HBOT (n= 27). In both groups, ^{131}I-MIBG

was administered at a dose of 200 mCu in the first treatment and 100 mCu in the subsequent ones. In the HBOT combination therapy group, HBOT sessions were performed 2 to 4 days after treatment with ^{131}I-MIBG, 4 to 5 consecutive days in multiplace hyperbaric chambers. The HBOT sessions profile included 12 minutes of pressuring the chamber from 1 to 3 ATA, followed by 75 min at 3 ATA. The decompression profile applied was from the *Canadian Forces Diving Manual* decompression tables. The O_2 was administered through a nasal/mouth mask, guided by transcutaneous optical O_2 tension to a target of 1000-1200 mmHg. The 28-month OS of patients undergoing ^{131}I-MIBG alone was 12%, whereas that of patients receiving combination therapy was 32%. Furthermore, HBOT was tolerated by all patients with no discomfort or increased toxicity.

The effectiveness of ^{131}I-MIBG combined with HBOT seems to be explained by the characteristics of neuroblastoma cells, as they have high levels of ferritin deposits and decreased activity of H_2O_2-detoxifying enzymes (catalase and glutathione peroxidase). The ^{131}I-MIBG itself is an inhibitor of complex I (NADH-ubiquinone-oxidoreductase), present in the mitochondrial electron transport chain, causing a leak of paired electrons, leading to increased production of superoxide radicals. Under normal conditions, superoxide radicals would be converted into hydrogen peroxide by the superoxide dismutase and, consequently, into water and O_2 by the catalase. As in neuroblastoma, the activity of the H_2O_2-detoxifying enzyme is reduced, and hydrogen peroxide accumulates intracellularly. In the presence of superoxide, hydrogen peroxide is converted into highly reactive hydroxyl radicals (conventionally named ROS) in the iron-catalyzed *Haber-Weiss* reaction, leading to the peroxidation of proteins, lipids, and DNA (50). In addition, the radiation effect is potentiated by HBOT, increasing the intracellular production of NOS and consequent increase in tumor cell damage (Figure 1).

8 Future perspectives

In this review, we sought to highlight the role and importance of HBOT as an adjuvant to some of the available neuroblastoma treatments, focusing on hypoxia and its role in oncobiology. It is clear that hypoxia acts as a trigger to several signaling pathways that regulate cancer growth. Still, it also induces significant changes in the TME (28) and, as such, should be considered for new treatment strategies.

As Borriello L et al. indicate, neuroblastoma is "truly a disease of the seed and the soil," in which the seeds can be identified as the neuroblastoma cells and the soil as the surrounding TME (20). The main question is to know to what extent the genetic modifications of these "seeds" can modify the "soil" to maintain its development and survival (20). This disease is driven not only by genetic events but also by the complex interplay between them and their influence on the TME through paracrine mechanisms, which significantly contribute to tumor progression and aggressiveness (20).

Stages III and IV of neuroblastoma represent some of the most difficult solid tumors to treat, and despite intensive research, progress toward a cure remains elusive (51). The main concern

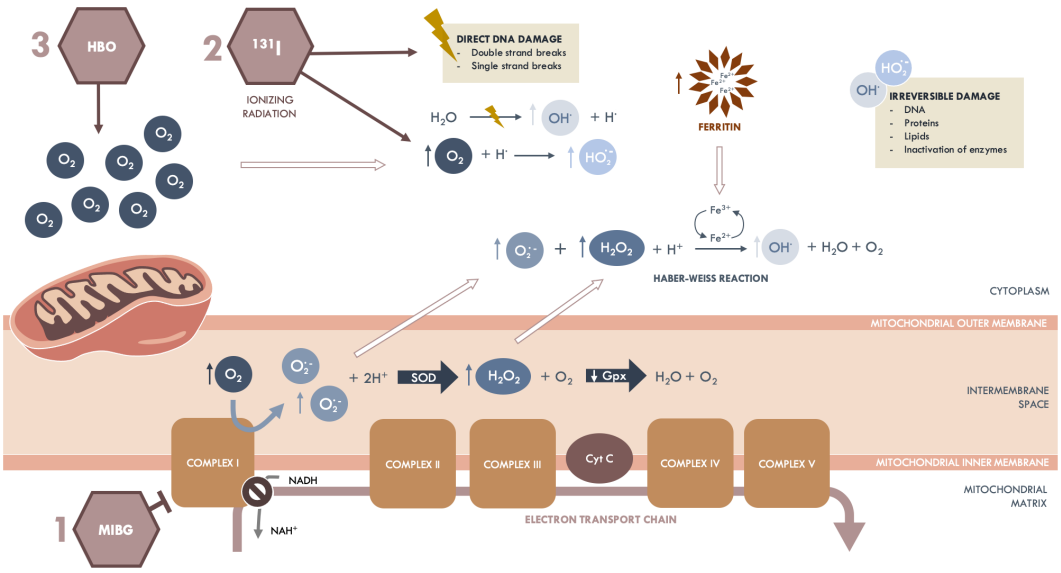

FIGURE 1
Effects of ^{131}I-MIBG combined with HBOT on neuroblastoma cells. The ^{131}I-MIBG has an antiproliferative effect by inhibiting complex I of the mitochondrial respiratory chain, leading to increased production of superoxide radicals. In neuroblastoma cells, the activity of H_2O_2 detoxifying enzymes is reduced, meaning that superoxide radicals are not converted into hydrogen peroxide and, consequently, into water and O_2. In the presence of superoxide radicals, hydrogen peroxide is converted to hydroxyl radicals in the iron-catalyzed *Haber-Weiss* reaction, leading to proteins, lipids, and DNA peroxidation. The ^{131}I emits ionizing radiation through the decay of the ^{131}I radionuclide, directly and indirectly affecting DNA damage. The indirect effect occurs through the formation of ROS by radiolysis of the water molecule, dependent on O_2. In turn, HBOT potentiates the effect of ^{131}I by increasing tumor O_2 levels. Cyt C, Cytochrome complex; Gpx, Glutathione peroxidase; HBOT, Hyperbaric oxygen therapy; $HO_2^{\cdot}$, Perhydroxyl radicals; H_2O, Water molecule; H_2O_2, Hydrogen peroxide; ^{131}I-MIBG, Meta-iodobenzylguanidine; $OH^{\cdot}$, Hydroxyl radical; O_2, Oxygen; $O_2^{\cdot-}$, Superoxide radical; ROS, Reactive oxygen species; SOD, Superoxide dismutase.

regarding high-risk, relapsed, metastatic, and/or resistant neuroblastoma is the increased tumor toxicity of aggressive cancer treatment and the enhanced risk of secondary tumors over the years. One of the strategies to reduce toxicity is to apply the respective treatment locally through nanotechnology. Both CTX and biologically targeted therapy or IMT may be more effective and have fewer side effects due to nanoparticle delivery (51).

Currently, the main treatments available can be summarized as follows: surgery, RT, CTX, facilitation of CTX via nanotechnology, stem cell rescue and myeloablation therapy, ^{131}I-MIBG, and IMT. Each of these approaches has its pros and cons. Particularly, multimodal therapeutic strategies and the resulting high toxicity and increased development of secondary malignancies after treatment must be carefully considered (51). Of the latter, IMT holds the most promise in controlling cancer progression, but significant challenges remain, most notably the several immune escape mechanisms employed by neuroblastoma cells (51).

Surprisingly, the combination of HBOT with IMT shows promise in increasing the efficiency of IMT, as HBOT can stimulate the distribution of programmed death-(ligand)1 checkpoint blockade, as well as the activity of tumor-infiltrated lymphocytes through extracellular matrix modulation. Furthermore, HBOT can reduce immunosuppression resulting from hypoxia, enhancing CD4+ lymphocyte activity (52).

Nanotechnology can potentially improve antitumor efficacy and reduce side effects, increasing patients' quality of life. However, translating from the bench to the bedside process is more complex than it might appear at first glance due to several factors, including mimicking the hypoxic TME (53).

Regarding early diagnosis and treatment monitoring, one of the areas of most significant interest is the evaluation of circulating tumor cells (CTC) and tumor DNA (ctDNA). Due to its discrimination ability to distinguish CTCs/ctDNA from primary tumors and distant metastases (by analyzing their chromosomal aberrations and/or DNA mutations), this technique is particularly relevant. Compared to adult solid tumors, neuroblastoma has fewer mutations, which presents a unique advantage. Moreover, it is possible to use single-cell and CTC proteomics analysis, which may lead us to a new step attributable to the capacity to detect cells more likely to resist treatment (54).

As cancer therapies keep evolving, incredible novelties, such as CAR-T cells, need to be highlighted, where several clinical trials in high-risk neuroblastoma are underway to evaluate this therapy´s potential. Yet, CAR-T cells in solid tumors do not seem to have a stellar impact, which the suppressive TME might explain. Therefore, there is a definite need to improve knowledge of TME in neuroblastoma to prevent immune system escape and increase the effectiveness of this therapy (55).

Hereafter, we expect that the investigation and understanding of these intricate interactions will undoubtedly guide new perspectives in clinical studies on neuroblastoma.

Author contributions

DAC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. JGN: Investigation, Validation, Writing – original draft, Writing – review & editing. MSA: Data curation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. NG: Investigation, Validation, Writing – original draft, Writing – review & editing. JAR: Data curation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. CEA: Supervision, Writing – review & editing.

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Acute and chronic central nervous system oxidative stress/toxicity during hyperbaric oxygen treatment of subacute and chronic neurological conditions

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Introduction: Oxygen toxicity has been defined as acute central nervous system (CNS), acute pulmonary, and chronic pulmonary oxygen toxicity. This study identifies acute and chronic CNS oxygen toxicity under 2.0 atmospheres absolute (ATA) pressure of oxygen. Methods: The authors' medical records from September 29, 1989 to January 20, 2023 and correspondence to the authors (9/1994 to 1/20.2023) from patients with signs and/or symptoms historically identified as acute CNS oxygen toxicity and those with neurological deterioration receiving hyperbaric oxygen for neurological conditions were reviewed. Acute cases were those occurring with ≤5 HBOTs and chronic cases >5 HBOTs. Chronic cases were separated into those at 1.5 ATA, > 1.5 ATA, or < 1.5 ATA oxygen. Cumulative dose of oxygen in atmosphere-hours (AHs) was calculated at symptom onset.

Results: Seven acute cases, average 4.0 ± 2.7 AHs, and 52 chronic cases were identified: 31 at 1.5 ATA (average 116 ± 106 AHs), 12 at >1.5 ATA (103 ± 74 AHs), and 9 at <1.5 ATA (114 ± 116 AHs). Second episodes occurred at 81 ± 55, 67 ± 49, and 22 ± 17 AHs, and three or more episodes at 25 ± 18, 83 ± 7.5, and 5.4 ± 6.0 AHs, respectively. Most cases were reversible. There was no difference between adults and children ($p = 0.72$). Acute intervention in cases (<3 months) was more sensitive than delayed intervention (21.1 ± 8.8 vs. 123 ± 102 AHs, $p = 0.035$). Outside sources reported one acute and two chronic exposure deaths and one patient institutionalized due to chronic oxygen toxicity. A withdrawal syndrome was also identified.

Conclusion: Hyperbaric oxygen therapy-generated acute and chronic cases of CNS oxygen toxicity in chronic neurological conditions were identified at <2.0 ATA. Chronic CNS oxygen toxicity is idiosyncratic, unpredictable, and occurred at an average threshold of 103–116 AHs with wide variability. There was no difference between adults and children, but subacute cases were more sensitive than chronic intervention cases. When identified early it was reversible and an important aid in proper dosing of HBOT. If ignored permanent morbidity and mortality resulted with continued HBOT.

KEYWORDS

oxidative stress, oxygen toxicity, hyperbaric oxygen, treatment, chronic neurologic conditions

1 Introduction

Oxidative stress/toxicity (OT) in humans is a function of partial pressure of oxygen and time of exposure (1) and is thought to be caused by damage from oxygen free radicals (2, 3). It has been shown to occur in nearly all tissues and organs (4). The most sensitive target organs are the lungs and central nervous system (1) (CNS: brain and spinal cord) and the manifestations are usually separated into acute toxicity from continuous exposure and chronic toxicity from repetitive exposures. Acute CNS OT from continuous exposure to oxygen was first described in animals in 1878 with hyperbaric exposures and is known as the Paul Bert Effect (5). Acute pulmonary OT from continuous exposure to oxygen was first described in 1899 with normobaric and hyperbaric exposures and is known as the Lorrain Smith Effect (6). Chronic or cumulative pulmonary OT from repetitive exposure at hyperbaric pressures has been described and led to the development of the unit pulmonary toxic dose (UPTD) metric in divers (7–9). Chronic or cumulative CNS OT from repetitive hyperbaric exposures has been implied and replicated in animals (10–12), but dismissed/attributed to other causes in humans (13–16). It has only been described from continuous exposures of hours to days and consisted of paresthesias and numbness in fingers and toes, headache, dizziness, nausea, and reduction in aerobic capacity (17).

The research and clinical experience on CNS OT under hyperbaric conditions has been conducted and reported at ≥2.0 ATA oxygen on neurologically normal subjects (18–20), or emergency indication patients (13–15). These studies report the extreme manifestation of acute CNS OT, grand mal seizures. Due to the low incidence of seizures at 2.0 ATA and the traditionally greater clinical levels of 2.36–2.4 ATA (13–15) CNS OT under hyperbaric conditions was considered non-existent or impossible to elicit below 2.0 ATA (21–26) or more recently 1.9 ATA (27). Even when obvious CNS symptoms developed from continuous exposure at 2.0 ATA "…the cause of the symptoms is not known" (22) or the symptoms were characterized as a "somatic" constitutional form of OT (28), omitting the descriptor "chronic" to avoid confusion with chronic pulmonary OT (29). The rectangular hyperbola pressure- continuous time exposure graph of acute CNS OT supports this belief (30) and has been reinforced by a lack of identification and reporting of cumulative CNS OT with repetitive HBOT under 1.9–2.0 ATA. This may be due to the reporting of CNS OT from the common clinical use of HBOT at pressures of 2.0 ATA or greater (31–39), but more likely is due to the lack of application of HBOT to chronic neurological conditions prior to Neubauer (40).

Based on Neubauer's work (40), the absence of reported CNS OT, and belief of non-existence of CNS OT below 1.9–2.0 ATA (21–27), Harch, Van Meter, and Gottlieb investigated the treatment of chronic neurological disorders with 40 treatment blocks of HBOT at 1.5 ATA/90 min daily from 1994 to 1999 (41). Cases with unanticipated untoward symptoms emerged in a group of carbon monoxide-poisoned patients with neuro-cognitive residual symptoms treated 6 months post carbon monoxide exposure (Reported at the Undersea and Hyperbaric Medical Society Gulf Coast Chapter Meeting 4/1995, New Orleans). Additional cases from multiple sources at exposures >1.5 ATA oxygen, prompted a cautionary statement to a worldwide parent support group for disabled children, the MUMS Network, in 1998 (reprinted 2022) (42) and a presentation of cumulative cases at the 2nd International Symposium on Hyperbaric Oxygenation and the Brain Injured Child in 1999 (43). In the subsequent 24 years the increasing neurological off-label use of HBOT by untrained medical and non-medical personnel, home use of hyperbaric therapy, continued contact of the primary author by patients with negative outcomes from HBOT for neurological disorders, and reports of permanent injury and death indicated a need for more widespread understanding of safe dosing limits of HBOT for chronic neurological disorders. This retrospective study addresses this need. Herein we report acute CNS OT within 5 HBOTs and chronic CNS OT with repetitive HBOT exposures, both at less than 2.0 ATA oxygen.

2 Materials and methods

Medical Records Archive and Search: The primary author maintained archives of all medical records of his hyperbaric patients from the time of the first application of hyperbaric oxygen therapy to divers with chronic decompression illness (September 29, 1989) and of all communications since 9/1994 from outside sources (physicians, hyperbaric medical facilities, families, and all individuals who sought consultation regarding hyperbaric oxygen therapy, particularly for complications of hyperbaric treatment) by phone, email, letter, or from a subsequent formal medical history and physical exam. The entire communication archive was searched from 9/1994 through Institutional Review Board approval of January 20, 2023 and a partial search of the medical records, mostly from the author's memory and separately archived cases with complications, was conducted from September 29, 1989 to January 20, 2023.

Type of Records Selected: Records were selected that featured signs and symptoms (SS) of oxygen toxicity described by Donald (19, 20) that occurred during treatment with HBOT for chronic neurological conditions. These SS were change in level of consciousness (loss of consciousness, drowsiness, sleepiness, amnesia, confusion, loss of judgment), neuromuscular signs (clonic spasms of the legs, "blubbering of the lips," muscle fasciculations, clumsiness, fibrillation of lips, lip twitching, twitching of cheek and nose or any muscle), salivation, behavioral SS (changes of behavior, fidgeting, disinterest), mood changes (depression, euphoria, apprehension, acute terror), autonomic SS (facial perspiration, diaphoresis, facial pallor, bradycardia, palpitations, sensation of arterial pulsation throughout the body, syncope); respiratory SS: panting, grunting, hiccoughs, inspiratory predominance, spasmotic respiration, choking sensation, epigastric tensions, epigastric aura, constriction or same in precordium; alteration of senses: paresthesias, visual symptoms (flashes of light, haloes, loss of acuity, lateral movement of images, decrease of intensity, constriction of visual field, hallucinations, micropsia), acoustic symptoms (music, bell ringing, knocking), hallucinations (unpleasant olfactory or gustatory sensations); malaise, spasmotic vomiting, vertigo, and SS in the acronym VENTIDS (visual, ear, nausea, twitching, irritability, dizziness, seizure). Synonyms were allowed, e.g., lethargy, lassitude, fatigue, and brady-kinesia for drowsiness or sleepiness.

Data Extraction, Analysis, and Statistics: Data from enrolled cases were extracted and a clinical vignette composed for each case. Data included age, sex, diagnosis, time from injury to HBOT, the HBOT schedule/dose, number of HBOTs at time of OT symptom/sign onset, the SS of OT, and cumulative oxygen dose at the onset of

SS. Cumulative dose was calculated in atmosphere-hours (AHs) with the following equation:

$$\text{AHs} = \text{Depth}\,(\text{ATA}) \times \text{Time of oxygen exposure}\,(\text{hrs.}) \times \text{Number of HBOTs.}$$

Cases were identified as the authors' or from outside sources and were sorted by their historical chronological occurrence. The first cases were observed at 1.5 ATA during the 1994 clinical trial (*vide supra*). They were segregated from cases treated at > or < 1.5 ATA oxygen. Chamber type was designated as hardshell (steel and acrylic, typically, with a pressure limit of 3.0 ATA or greater) or softshell (synthetic soft material composition and pressure limits of 1.3 ATA). Functional brain imaging was included when available. SPECT scanner specifications, radiopharmaceutical, acquisition, and processing are described in Supplementary File S1 (44, 45). This study was approved as LSU IRB #4574. GraphPad[1] was used to perform unpaired t tests on group comparisons. Means were calculated using Calculator.net.[2]

3 Results

Eighty-two cases were enrolled. Twenty-two cases were excluded due to equivocal signs and symptoms, critical missing data, or attribution to alternative explanations of SS, leaving 60 cases for the final cohort. These comprised 27 cases from a previous IRB-approved study and 33 cases since 7/2001. The study was closed at 60 cases, arbitrarily judged by us to be sufficiently representative of chronic CNS OT.

Abbreviated histories of all cases are in Supplementary File S2. Case reports from outside sources used the mother, family member, or immediate caregiver's quotation of signs and symptoms. During the review acute CNS OT cases were identified from outside clinics. Five HBOTs were chosen as the cutoff for acuity (32–35). Included in the chronic cases were patients who demonstrated initial improvement in neurological and cognitive function with HBOT then reversed these improvements with continued HBOT, re-expressing their neuro-cognitive deficits, and finally regained their HBOT- induced improvements after discontinuance of HBOT. This pattern strongly suggested the cumulative oxidative stress with HBOT and recovery from same on discontinuance of HBOT described by Bean and Siegfried (46). The pattern was also indicative of typical drug overdosing and recovery upon discontinuance of the drug. As a result, this pattern was identified as a CNS OT manifestation.

Data from the Supplementary File S2 case reports are presented in Supplementary Tables S1–S5 and in Supplementary Table S1 (acute oxygen toxicity), Supplementary Table S2 (chronic CNS oxygen toxicity at 1.5 ATA oxygen), Supplementary Table S3 (chronic CNS oxygen toxicity at >1.5 ATA oxygen), Supplementary Table S4 (chronic CNS oxygen toxicity at <1.5 ATA oxygen), and Supplementary Table S5 (single case of withdrawal syndrome). Data from Supplementary Tables S1-S4 are condensed in Table 1.

Supplementary Table S1 contains 7 cases of acute CNS OT, most of which (5 cases) occurred at ≤2.0 ATA. They were 2–95 y.o., 6 males, 1 female, with 7 different neurological diagnoses (two with seizure disorders). Five of the cases were in the chronic disease phase (greater than 6 months from time of injury, insult, infection, diagnosis). The single mortality was a 95 y.o. male with 3,000 previous HBOTs for a stroke who was treated a few hours after an acute stroke, experienced CNS OT on the second treatment, a grand mal seizure on the 3rd HBOT (1.1 ATA oxygen), 15 subsequent grand mal seizures in the next 24 h, and died after the 15th seizure.

Supplementary Table S2 lists 31 cases of chronic CNS OT after >5 HBOTs and at 1.5 ATA oxygen that include the first two unrecognized cases treated by the authors in 1990 and 1992 (47). The patients were 18 months to 60 y.o., 19 M, 12 F, 4 weeks to 10 years post injury/insult (29 ≥ 6 months since injury/insult), with 13 different neurological diagnoses. There were two severe complications from outside sources occurring in home hardshell chambers (one death and one institutionalization for severe behavioral deterioration). One case's OT (#16) was serendipitously captured on SPECT brain blood flow imaging (Figure 1) in an IRB-approved study of HBOT in chronic severe TBI. There were nine cases with second episodes of CNS OT and six cases with three or more episodes.

Supplementary Table S3 contains 12 chronic CNS OT cases occurring at >1.5 ATA oxygen pressure. There were 9 males, 3 females, ages 2–58 y.o., 8d to 12 years post injury/insult, with 9 different diagnoses. There was one death from an outside source in a home hardshell chamber. The OT in six of 12 cases occurred after an escalation of oxygen pressure or on intensive twice/day or 6–7d/week schedules. Seven of the 12 cases occurred at 1.75 ATA, one at 1.55 ATA at 6,900 feet altitude, two at 2.0 ATA, one at 2.4 ATA, and one at 2.8 ATA. Second episodes of OT were documented in 5 cases and three or more episodes of CNS OT in one case.

Supplementary Table S4 contains nine chronic CNS OT cases occurring at <1.5 ATA oxygen pressure. There were 4 males, 5 females, ages 2.5–83 y.o., 2.5 months to 11 years after injury/insult with seven different neurological primary diagnoses. Four cases of OT occurred in portable chambers, two of whom occurred after prolonged chamber treatments (2–3 h each) or a prolonged course of treatment (135 HBOTs), and a fifth with post-surgical normobaric oxygen after long-term HBOT in a portable chamber. One was a subacutely drowned child who could not tolerate 1.15 ATA/45 min of compressed air. Overall, 4/9 had at least one episode while receiving compressed air at ≤1.5 ATA. Second episodes of CNS OT were documented in five cases and three or more episodes occurred in 3 patients.

Supplementary Table S5 lists a single case of apparent drug withdrawal that occurred after 130 HBOTs, the last 70 of which were consecutive, 7d/week, twice/day treatments. In the subsequent 2 weeks she experienced regression of her gains followed by a marked worsening of her pre-HBOT dystonia, involuntary total body movements, and teeth-grinding.

The critical data from Supplementary Tables S1-S4 are condensed in Table 1. The acute and chronic cases occurred over similar wide age

1 GraphPad.com

2 https://www.calculator.net/standard-deviation-calculator.html

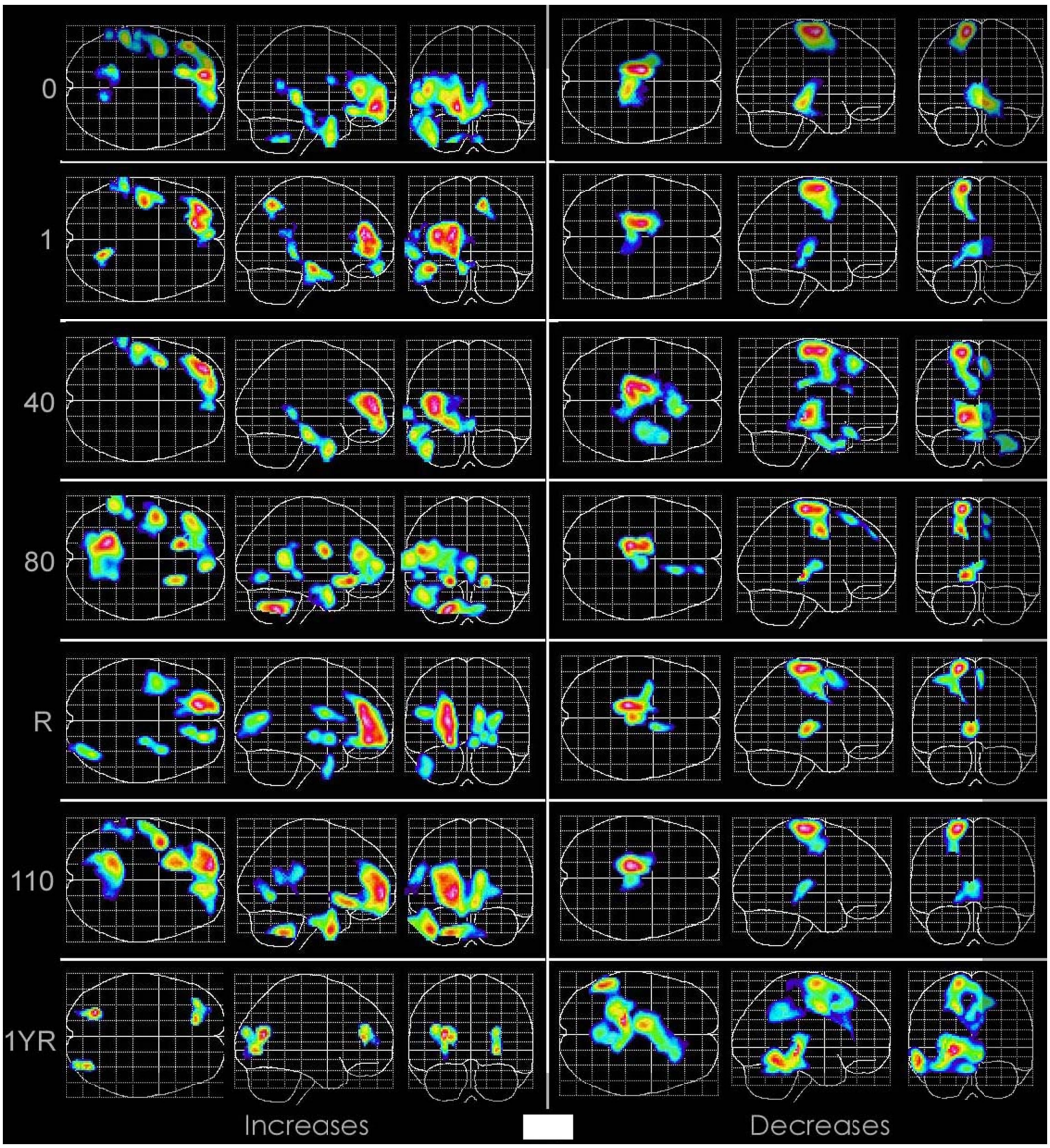

FIGURE 1
Sequential SPECT brain imaging of Subject #16 before the first HBOT (Row 0) then after 1, 40, 80 HBOTs, a 6-month break (Row R), 110 HBOTs, and 1year post 110th HBOT (1 YR). Images are statistical parametric maps of selective coregistered slices. Color scheme: black indicates rCBF is within reference normal range. Increasing Z-scores (blood flow) are blue, green, orange, red, white on the left 3 columns and decreasing Z-scores with the same color scheme on the right 3 columns. SPECT at time 0 shows multiple areas of abnormally increased brain blood flow (top row left 3 images) and abnormally decreased brain blood flow (top row right 3 images) compared to normal and the other 6 patients in the study. Note intensification and broadening of Row R increased right frontal blood flow (6months after 80th HBOT) that shows persistence and increases in the right frontal, posterior fronto-parietal and parietal, opposite anterior frontal, right temporal, and cerebellar areas after removal from the study for behavioral deterioration (Row 110), nearly all of which recede 1year post study (1YR) to less than pre-HBOT levels (Row 0). Simultaneously, at 1YR post HBOT the right 3 panels show broadening of the adjacent areas with significant decreases in blood flow compared to the pre-HBOT level. Reproduced with permission Best Publishing Company (43).

ranges. Acute cases experienced CNS OT at <5 AHs while the chronic cases occurred regardless of pressure over a wide range of cumulative oxygen exposures with mean of 103–116 AHs. Second and third episodes of chronic CNS OT occurred with lesser additional cumulative AHs, markedly so in the cases at <1.5 ATA oxygen (approximately at 1/5th the AHs of the first episode).

Two comparisons were performed on the chronic CNS OT cases: (1) First episode in children (107 ± 94 AHs; case #49 omitted from calculation per above) vs. adults (117 ± 106): found to be non-significant ($p = 0.72$), and (2) Delay to treatment, based on start of continuous treatment that resulted in CNS OT. Subacute, ≤ 3 months (Cases #27, 48, 50, 57, 58: 21.1 ± 8.8 AHs), were compared to chronic cases, > 3 months [all other cases in Supplementary Tables S1-S4, except case #49 (46 cases)]: 123 ± 102 AHs, $p = 0.035$.

4 Discussion

Using well-identified SS of acute CNS OT, 52 cases of chronic CNS OT from outside and internal sources are presented in this study.

TABLE 1 Acute and chronic CNS OT essential data from Supplementary Tables S1-S4.

	Oxygen pressure at occurrence of OT (ATA)	Number of cases; gender (% M, F)	Mean age ± (SD)	Mean AHs ± (SD) 1st OT	Mean AHs ± (SD) 2nd OT	Mean AHs ± (SD) ≥ 3rd OT
Acute O_2 toxicity	1.5	1; F	32	3	–	–
	1.5 ≤ 2.4	5; M (100%)	24.6 (23.4)*	4.7 (3.0)	–	–
	<1.5	1; M	95	1.7**	0.36	–
Chronic O_2 toxicity	1.5	31; M (61%)	30.3 (22.6)*	116 (106)	81 (55)[&]	25 (18)
	1.5 < 2.8	12; M (75%)	19.2 (16.3)	103 (74)	67 (49)	83 (7.5)
	<1.5	9; M (44%)	30.8 (25.5)	114 (116)	22 (17)	5.4 (6.0)

AH, Atmosphere Hours of oxygen exposure; OT, Oxygen Toxicity; SD, Standard Deviation. *Age was estimated on two subjects where exact age was unknown. **Patient with cumulative 4052 AHs upto few days before acute stroke then oxygen toxicity after 2nd subsequent HBOT. [&]Excludes one outlier.

For comparison we also gathered 7 cases of acute CNS OT from ≤5 HBOTs. These 59 cases have demonstrated that: (1) The historical rectangular hyperbola of pressure/time continuous O2 exposure for CNS OT in normal people does not apply to patients with acute or chronic neurological conditions, (2) Acute CNS OT can occur at <2.0 ATA oxygen, (3) Chronic CNS OT exists and can occur at non-traditional lower pressures (<2.0 ATA oxygen and < 100% FiO2); (4) CNS OT can occur in chronic neurological conditions at very low doses, such as compressed air at 1.3 ATA, < 100% FiO_2 at 1.3 ATA, and even 1.15 ATA of compressed air in both portable and hardshell chambers; (5) Both acute and chronic CNS OT at <2.0 ATA occurred at 103–116 AHs, although there was wide variability consistent with the historical finding of acute CNS OT at higher pressures; (6) There was no difference in sensitivity between adults and children, but subacute intervention resulted in earlier CNS OT than delayed intervention; and (7) CNS OT was reversible if recognized early, but resulted in permanent morbidity and mortality if treatment persisted.

All cases were separated initially by pressure dose of HBOT at first occurrence of OT (cases at 1.5, > 1.5, and < 1.5 ATA oxygen and with less than 100% FiO_2 oxygen) based on their historical presentation in our initial clinical practice and then an IRB-guided study on HBOT in chronic neurological conditions using 1.5 ATA oxygen. We found that this historical separation was arbitrary. While there was a wide range of oxygen sensitivities at a given oxygen pressure consistent with Donald's findings (19, 20), chronic CNS OT occurred at a nearly equal average cumulative oxygen dose (116, 103, and 114 AHs), regardless of oxygen pressure. Many of these cases (#'s: 8,9,11,12,13,14,15,17,18 ,20,21,22,23,25,27,34,37,39,40,41,43,44,45,46,48,50,52,53,55,59) occurred after prolonged courses of treatment and/or from intensive twice/day or 6–7d/week schedules in monoplace chambers that commonly were not equipped or did not use air breaks, a well-proven method for avoiding oxygen toxicity (48–51). Twenty-one percent occurred after an increase in oxygen or pressure dose (case #'s: 22,27, 39,40,41,42,46,51,52,53,57). This was even manifest at very low doses such as pressurized air where CNS OT was experienced after increases in chamber pressurization time to 2–3 h. Regardless of the dose at which CNS OT occurred, the CNS OT was mitigated in some cases (Case #'s: 17,19,22,46,53,55,56) by a decrease in oxygen exposure (decrease in intensity of schedule or reduction in dose) similar, again, to the well-proven effects of air breaks (48–51).

Our study also found that repetitive episodes of CNS OT occurred at lesser doses of oxygen consistent with animal studies which have shown sensitization to CNS OT after an initial episode (52). For the cases at 1.5 and > 1.5 ATA oxygen the second episode of CNS OT occurred at similar amounts of additional AHs, 81 and 67, respectively, and for those at 1.5 ATA the third or greater episode of CNS OT after 25 AHs. At >1.5 ATA there was only a single case of 3 or more episodes of CNS OT and so did not afford a comparison to the other oxygen pressures. At less than 1.5 ATA oxygen the second episode was experienced after 22 additional AHs, and the third or greater episode after 5.4 additional AHs. These lower numbers suggested an idiosyncratic hypersensitivity, reflecting Donald's findings of wide idiosyncratic variability in oxygen sensitivity (19, 20).

Our study identified one case (case #60) of an habituation effect to HBOT where shortly after cessation of a prolonged intensive course of HBOT there was a notable deterioration in the patient's condition to a level worse than the pre-HBOT level. This phenomenon was characteristic of a withdrawal syndrome seen with habit-forming drugs (53). Unfortunately, there was no long-term follow-up information on this case.

If CNS OT was recognized early and accommodated no significant lasting clinical injury was apparent. This comports with the widespread clinical impression of no harm from even the most severe form of CNS OT, seizures (1, 20, 54). However, Bert (5) believed that a toxic chemical substance was responsible for the OT seizures and Bean and Siegfried (46) conceived that the toxic substance of "transient or minute quantity" might still induce tissue injury (46). This transient toxic substance of minute quantity is now known to be reactive oxygen species (2, 55). ROS/oxidative stress have been shown to occur with single HBOT exposures at both 1.5 and 2.4 ATA (56). ROS and their by-products in OT seizures in animals have demonstrated permanent effects, including lipid peroxidation (3), apoptosis (57), cognitive injury (58), motor deficits (46), and ischemic lesions (11). Similar to Bean and Siegfried's description (46), if oxidative stress continued in the setting of SS of CNS OT lasting injury occurred. In our series this included two deaths and in a third case who required institutionalization due to severe behavioral deterioration.

Bean and Siegfried (46) methodically investigated both acute and chronic CNS OT in animals, using repetitive (3–4x/day), multi-day 5.42 ATA oxygen exposures. Their findings included: (1) OT reactions during decompression and post-decompression could be described by acute and chronic phases, (2) Acute toxicity occurred dominantly during decompression or post-decompression and was idiosyncratic with a wide variation in individual susceptibility, (3) The intensity and duration of acute reactions showed wide variation and recovery was rarely instantaneous or immediate, (4) The susceptibility to seizures

was increased by successive exposures and the time to occurrence of premonitory symptoms decreased with repeat exposures, (5) Successive exposures increased the severity and prolonged the duration of the acute post-decompression reactions such that lengthening the surface interval or stopping the exposures for a day or more was necessary for recovery, (6) Seizures began most commonly during decompression, (7) Individual sensitivity to the acute and chronic manifestations was unpredictable and empiric, (8) Acute decompression effects eventually merged with chronic effects which developed gradually and upon which repeat acute reactions were layered, (9) The outstanding feature of the chronic phase was motor manifestations that were permanent, (10) Augmentation of chronic effects could be induced by increasing the duration of the oxygen exposure or decreasing the surface interval, (11) In less susceptible animals the surface interval was important: increasing the frequency from 3 to 4x/day hastened the onset and decreased the total number of exposures necessary to bring about chronic alterations, (12) A few animals that were resistant to seizures became lethargic and anorexic, (13) The reactions to O2 were generally reversed by lowering the oxygen pressure or returning to normal atmospheric pressure.

Nearly all of the above findings of acute and chronic effects of OT are exhibited by the patients described in our study's clinical vignettes. The character and development of these patients' adverse SS are described by finding #'s 1, 2, 3, 4, 5, 7, 8, 10,11, 12, and 13. The primary differences between our cases and Bean and Siegfried's (46) animal cases are: (1) the initial manifestations were often more subtle, likely due to the lower doses of HBOT used, but accumulated with repetitive exposures, similar to what has been described in large reviews of patients developing oxygen toxicity seizures during courses of HBOT for standard indications at higher doses (31–36), (2) seizures occurred both during the treatment and in-between treatments, and (3) the outstanding features of the chronic phase were not only motor, but behavioral, emotional, affective, and other. While not tested by Bean and Siegfried (46) we also found no difference in development of chronic CNS OT between adults and children.

In addition, all of our study's cases had neurologic comorbidity, implying an increased sensitivity to CNS oxidative stress with neurologic pathology. This sensitivity was more apparent in the patients with subacute neurologic injury where OT occurred at only 20% of the first episode cumulative AHs of patients with >3 months post injury. It suggests an augmentative effect of ROS on recently injured/inflamed neural tissue. The sensitization effect of neurologic comorbidity on CNS OT is consistent with the major review by Heyboer et al. (35), however, our cases occurred at a greater average cumulative oxygen exposure, 111 AHs, than the cumulative oxygen exposure (61 AHs) for the most severe form of oxidative stress, CNS OT seizures, in six large series of patients with mostly chronic HBOT wound indications treated at the typical 2.4 ATA/90 min (31–36). This is likely due to the higher pressures used in these series. These pressures and durations of exposure are near the inflection point in the rectangular hyperbola (30) of pressure/time continuous exposures for increased manifestation of CNS OT. Oxidative stress is proportional to pressure and time of exposure with mitigation by air breaks and surface intervals. With these greater pressures there is an increased level of oxidative stress with each treatment and cumulatively greater levels of oxidative stress require greater times to decay in both CNS (46) and pulmonary OT (1, 9). Given the similar air break (surface interval) of 22–23 h in a once/day treatment in most of our and the large series' cases it is likely that there is retained injury from oxidative stress with these higher pressure treatments that has not been mitigated by the time of the next daily.

TREATMENT. This would be consistent with the kindling effect of repetitive exposures to hyperbaric oxygen (10) and the autocatalytic nature of oxidative stress (49) where these repetitive higher pressure exposures likely reach the auto-catalytic threshold sooner than patients exposed to the lower pressures of oxygen. This, again, reinforces Bean and Siegfried's (46) proposal of transient effects of HBOT exposures and oxidative stress as well as permanent (and cumulative) effects which have been validated by others (10, 12).

Neurologic comorbidity in our subjects may also explain the most important finding of our study, that the rectangular hyperbola pressure/time CNS OT graph with its 2.0 ATA oxygen asymptote does not apply to patients with cerebral disorders receiving repetitive exposures to HBOT. The rectangular hyperbola misled us and the medical community into the false notion that CNS OT could not occur under 2.0 ATA oxygen. The central flaw in this notion was that the curve was generated from continuous hyperbaric oxygen exposures to normal people, mostly young male military members. We speculate that the human central nervous system in normal young males has the anti-oxidant capacity to equilibrate the oxidative stress of a continuous exposure to oxygen at less than 2.0 ATA, but not above. We further speculate that had seminal researchers in CNS OT (18–20, 22) exposed neurological patients to the same continuous exposures a typical hyperbola with both y and x axis asymptotes would have likely resulted. This is what our data describes with CNS OT occurring across a range of oxygen pressures from 2.0 to 0.24 ATA oxygen which approaches an x axis pressure asymptote. The only difference is that the autocatalytic threshold appears to be in the range of 103–116 AHs of cumulative oxygen exposure in chronic neurologic patients and far less with subacute/acute neurologic disease.

In one case CNS OT was captured on functional brain imaging that tracked the clinical manifestations and recovery (Figure 1). The acute manifestations were consistent with what has been identified with CNS OT in animals, increased brain blood flow prior to seizures (59). Despite the clinical improvement in the patient, the final imaging suggested negative residual effects with areas adjacent to the toxic areas showing significant reductions in blood flow below normal. Interpretation of this finding is difficult since the area of toxicity (high activity) would be expected to be the area showing OT injury (*vide supra*), not the adjacent areas unless there is an ischemic blood flow steal phenomenon from the adjacent areas to the high flow areas during the toxicity period.

An unexpected and derivative implication of the similar SS of CNS OT regardless of pressure and FiO_2 of oxygen is that increased pressure and hyperoxia are bioactive across the range of pressures from 0.24 to 2.0 ATA. This bioactivity, particularly for the patients who expressed SS of CNS OT at 1.3 ATA of compressed air, is additional proof that subjects receiving compressed air cannot be a control group in hyperbaric medicine clinical trials. The claim in HBOT cerebral palsy studies (60, 61) and mTBI PPCS studies (62–65) is that compressed air control groups are placebo groups. A placebo must be inert (66). If compressed air was inert it would be impossible for the subjects in our study to have experienced CNS OT.

4.1 Caveat

This study requires comments on the secondary issues that generated consternation in the review process and that are certain to cause consternation in some readers. Documentation of CNS OT in our cases necessitated presenting the case histories of the patients. These histories frequently reported symptomatic improvement of chronic neurological conditions before the manifestations of CNS OT and neurological deterioration. Accepting the toxicity events implies a concomitant tacit acceptance of the reports of neurological/cognitive improvement in the patient's disorders, all of which were off-FDA label diagnoses and nearly all of which were treated with atypical pressures, durations, and numbers of treatments. This study was not intended to be an indirect endorsement of off-label use of HBOT. It intended to report a series of inexplicable clinical observations that at first were unrecognized then later appreciated as reproducible/common. These clinical phenomena contradicted decades of an unchallenged tenet on the impossibility of CNS OT at less than 2.0 ATA oxygen. They refuted not only this false tenet, but exposed the flawed confused foundation of the modern field of hyperbaric medicine (67) and the atypical, idiosyncratic, and arbitrary FDA-"approval" process applied to HBOT. The flawed foundation and FDA- "approval" of HBOT has led to the resurrection and expansion of 300 previous years of "off-label" use of HBOT seen in our cases and with the proliferation of home hyperbaric chambers.

FDA-"approval" or FDA-"label" (clearance of a drug or device for marketing in the United States) is synonymous with "scientifically proven" and "off-label" with "unproven" and "unscientific." FDA-"approval" was historically predicated on randomized clinical trials. Not so for HBOT. In 1979 the FDA grandfathered "approval" of 13 diagnoses (68) for HBOT (the FDA-"label") based on a Delphi Consensus of opinion (67) of a group of hyperbaric physicians that were members of the Hyperbaric Oxygen Therapy Committee of the Undersea Medical Society, now the Undersea and Hyperbaric Medical Society (UHMS). While not without clinical evidence 12 of the 13 diagnoses had no randomized trials to support their use (67), 7 of the 12 still do not, and two of the last three diagnoses added to this list by the UHMS and endorsed by the FDA without formal application similarly have no randomized trials to support their "approval" (67). One of these three, intracranial abscess, was "approved" based on 20 worldwide cases, 14 of which were reported in a case series and the additional six from a medical society call-out over 5 years for treated cases (69). The second, central retinal artery occlusion, was also approved without a single randomized trial (67, 70). In a meeting with this author in 2/2002 on behalf of the International Hyperbaric Medical Association to request evidence requirements for new FDA-"approved" HBOT indications the Devices and Radiological Health Section of the FDA admitted that the 13 indications lacked clinical evidence. To this day the main problem with new FDA "approved" indications is their dependency on the same Delphia Consensus of the UHMS HBOT Committee where the flawed definition of HBOT (67) precludes acknowledging the scientific proof for a number of the off-label indications seen in our study. The result is off-label use for some indications that have greater proof than most of the existing 15 indications (60, 71–73).

The flawed unscientific historical definition of HBOT declared that HBOT was the use of 100% oxygen at >1.4 ATA for a list of "certain recalcitrant, expensive, and otherwise hopeless medical problems" (74) determined by Delphi consensus. "Certain," however, has led to a similar Delphi Consensus list of 23, 48, and 20 diagnoses in Russia, China, and Japan (67). Any pressurization under 1.4 ATA with 100% oxygen or < or > 1.4 ATA with less than 100% FiO2 was undefined and not hyperbaric oxygen. This begged the question, "What was it?" This entire excluded pressure and FiO2 range was the domain/basis for the 300 previous years of use of pressurized air therapy, including the introduction of hyperbaric medicine to the U.S. during the Spanish Flu Pandemic where pressurized air was used to resuscitate dying Spanish Flu patients (75). It was the bioactivity of pressure and hyperoxia along the spectrum of pressures and FiO2s > or < 1.4 ATA and < 100% O2. In particular, the bioactivity of <1.4 ATA 100% oxygen was considered by the clinical hyperbaric medicine field to be non-existent such that it could serve as a placebo control in hyperbaric studies. Supplementary Table S4 cases in this study refute this placebo control notion by demonstrating CNS OT at less than 1.4 ATA 100% oxygen, i.e., bioactivity of pressure and oxygen <1.4 ATA. If treatment in these pressure ranges were a placebo they could not generate toxicity effects. The fact that they did, consistent with the CNS OT SS at much higher doses, reaffirmed this bioactivity and refuted the non-scientific definition of HBOT.

In 2011 the FDA elucidated the flawed definition of HBOT by identifying HBOT as a dual component therapy consisting of increased pressure and hyperoxia (76). Their declaration was confirmed by 50 years of elegant physiologic studies that demonstrated the ubiquitous sensitivity of all living organisms to barometric pressure (77). Unappreciated by the clinical hyperbaric medicine field a critical review documented biosensitivity to barometric pressure in the 1.0015 to 1.3 ATA range, the identical range of the portable chambers that have proliferated in the past 20 years (77). This literature reinforced the FDA's classification and a scientific understanding of hyperbaric oxygen therapy for the first time in 347 years.

The FDA understanding also informed the proliferation of off-label use of HBOT that began in 2001with a series of randomized "controlled" HBOT studies in acute stroke (78) and cerebral palsy (60) (CP). Rusyniak et al.'s (78) acute stroke "control" group received 1.14 ATA oxygen (a purported inert placebo control because it was outside the traditional definition of HBOT) and achieved statistically significantly better outcomes than the 2.5 ATA oxygen group, outcomes identified as "excellent" by multiple prominent stroke researchers (79). In Collett et al.'s (60) CP study 1.3 ATA air was used as a control treatment and achieved equal statistically significant improvements compared to the 1.75 ATA oxygen group in a placebo-insensitive objective measure, Gross Motor Functional Measures. Both groups also experienced cognitive improvements that have never been seen with other therapies for CP. The HBOT improvements in GMFM exceeded the improvements in GMFM for all traditional therapies for CP except dorsal rhizotomy of the spinal cord (72). HBOT was declared ineffective due to the flawed definition of HBOT identifying the lower dose HBOT groups as placebo control groups. However, the scientific basis for the positive data in these "control" groups was reinforced by a series of low pressure low FiO2 animal and human studies performed in Japan (80), while the definition-based design flaws were later reproduced in a series of U.S. DoD mTBI studies beginning in 2013 that repetitively used 1.2 and 1.3 ATA air groups as placebo controls (62–65). When the pseudo-control groups experienced equal or greater large effect size (81) symptomatic and cognitive improvements compared to the higher pressure oxygen

groups the studies were again concluded to show the inefficacy of HBOT based on the flawed definition of HBOT. A scientific systematic review of these studies according to pressure and hyperoxia dosing of HBOT in mTBI has refuted these erroneous conclusions (71). The lay public, acting on these studies and responding to aggressive marketing by portable chamber manufacturers, began experiencing the results achieved by Collet in CP (60), Japanese researchers (80), the DoD TBI studies (62–65), and others (82) in pediatric and adult neurological disorders. It is this data backed by the science that has fueled off-label use and the proliferation of portable chambers for "scientifically unproven" off-label uses. Of the 60 cases in this study 30 (9 CP, 17 TBI, and 4 stroke) are for the "scientifically unproven" uses investigated in the above randomized trials. An additional 9 patients (5 with decompression illness and four with chronic carbon monoxide poisoning) were treated with HBOT based on clinical findings evident in the treatment of patients with these acute and subacute "approved"/"proven" indications. In essence the off-label use of HBOT in the U.S. is the lay public responding to the scientific evidence of bioactivity of pressure and hyperoxia in published studies and seeking treatment of their conditions based on this science. Because of the absence of an FDA label and inconsistency with the flawed definition of HBOT they remain "unproven."

Irrespective of the science and non-science, the second major issue of this caveat is that HBOT is a prescription medical treatment that can and has been abused. Currently, anyone from a businessman to massage therapists in "spas" can purchase a hyperbaric chamber either used or on prescription from a hyperbaric chamber company physician who sight unseen will provide the prescription to any untrained person without oversight. The disturbing deaths of two children in this study and institutionalization of an adolescent from overtreatment were avoidable. They resulted from placement of hardshell chambers in homes where they were operated by untrained parents. As can be seen in Supplementary Table S4, however, complications also occurred in portable chambers at lower pressure. A hyperbaric chamber has a reasonable safety profile when used properly, however, it is not without risk, especially when high FiOj is used. While benefit seems to be accruing from expanding clinical studies and widespread public use of chambers the true incidence and prevalence of chronic CNS OT from HBOT is unknown and likely suppressed by factors peculiar to this genre of treatment. Many of the cases in this report were communicated to the authors as a sole outlet for questions regarding the poor outcome of their family member. Acting on published data, many of the families sought HBOT over objections from their primary physician or neurologist. After a poor outcome occurred there was often no physician at the hyperbaric facility to consult or physician-associated institution to whom to register a complaint. Businessmen/owners of the facilities often told the families that the outcome was inexplicable, that "everyone normally gets better." In one case the North Carolina owner of the clinic threatened the mother with legal action and removed her from the facility's online chat room over her complaints of new onset seizures of her child in the chamber. Family members or parents felt that they took a risk and the consequences were theirs. Case in point was a drowned child who died in a Florida hyperbaric chamber in 2020. The child's mother lamented on social media that "I failed him (my baby), I will blame myself forever, I took the risk" (83). Safe use of hardshell chambers by the lay public has been accomplished in the UK (84) where centers were originally operated by the lay public under a non-profit trust with now de-prescriptioned 100% oxygen. It is intended that the descriptions of CNS OT in this study's clinical vignettes will inform the medical and non-medical community of more appropriate dosing of HBOT in chronic neurological disorders and avoid the avoidable complications documented in this report. It has done so for us and allowed more nuanced and personalized treatment of patients.

In conclusion, this research study has satisfied the two purposes of research, it has answered a question and posed more questions for future research than it answered. Recommendations based on this study are for continued expanded research of HBOT indications, acceptance of the understanding of HBOT as a dual-component therapy with bioactivity across a wide range of pressures and FiO2's, and for delivery of this therapy under physician oversight and guidance as a practice of medicine. We do not recommend the use of rote protocols for any diagnosis since medicine outside of experimental protocols is not protocol-driven, but rather individually delivered, as it has been for thousands of years. It is the use of rote protocols that has led to the widespread use/abuse of this therapy by non-medical professionals and many of the complications compiled in this report. Those complication occurred from lack of recognition/ignoring deterioration in the patient's condition while under HBOT treatment (no medical training or expertise) and recommending extended treatment times in the chamber based on the myth that "more is better."

5 Limitations

Limitations of this study include its retrospective nature and inability to verify cases from outside sources with medical records or direct observation as in the authors' cases. However, verification was discounted due to the context of the cases: parents of special needs children are keen observers, very familiar with their childrens' neurological deficits, and the parents alarm occurred in the setting of expected beneficial outcomes. Deviations from these expectations were easily observed. Some data is missing and details of "neurological," "cognitive," "symptomatic," and "physical" "improvements" are inexact in some instances. These omissions and inaccuracies were felt to be minor because the most important part of the vignettes were the patterns and negative SS exhibited by the patients which were similar. The patterns were either a neurological reversal after neurological improvement or development of SS of CNS OT identified for acute CNS OT that worsened as HBOT continued. Both the neurological reversal and CNS OT SS could not be attributed to any other cause and they recovered after discontinuation of HBOT, similar to acute CNS OT.

Lack of objective measures of oxidative stress/toxicity is a major limitation. Documentation with biomarkers would have strengthened the study. Only one instance of biomarker documentation was serendipitiously captured with SPECT brain imaging (Figure 1). Before OT biomarkers were available CNS OT was documented by observation of signs and reporting of symptoms. The list of these identified SS are very broad and amount to oxidative stress at almost any anatomical site in the CNS. The absence of biomarkers in this

study is a distinct flaw, but one that is intrinsic to the historical habit of hyperbaric CNS OT documentation. Given the non- prospective nature of this study documentation with biomarkers would have required pre/post sampling which was impossible in a retrospective study. While many biomarkers of OT are available today (85–90), none are routinely used in clinical hyperbaric medicine practice, and the results are mixed in their use in clinical hyperbaric oxygen studies (91). Future clinical studies on hyperbaric CNS OT should employ biomarkers.

Other limitations include a possible contribution of increased barometric pressure and oxygen vasoconstrictive effects. A contribution of increased barometric pressure to what has always been assumed to be the effects of oxygen pressure alone are possible. Even slight increases in pressure are bioactive for nearly all living organisms (77), but any contribution to the observed SS in our cases is impossible to parse or assess in our study. Oxygen vasoconstrictive effects might explain some or all of the CNS OT SS in acute cases 4, 6, and 7 of Supplementary Table S1, but would seem unlikely in the 52 chronic cases where ischemic deterioration would be expected in the chamber on each HBOT. This is not what we observed or families reported.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Louisiana State University School of Medicine Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because It was a retrospective chart review and due to the time over which the records were reviewed it would have been impossible to contact all of the subjects. The research would have been impossible to perform without the waiver.

Author contributions

PH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. SR: Data curation, Methodology, Project administration, Resources, Writing – original draft.

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Conflict of interest

PH is the owner of an S Corporation that is the vehicle for his practice of hyperbaric medicine. He also performs consulting and gives expert witness testimony under this S Corp.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2024.1341562/full#supplementary-material

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Using the gross motor function measure evolution ratio to compare different dosage of hyperbaric treatment with conventional therapies in children with cerebral palsy – could it end the controversy?

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The Gross Motor Function Measure is used in most studies measuring gross motor function in children with cerebral palsy. In many studies, including those evaluating the effect of hyperbaric treatment, the Gross Motor Function Measure variations were potentially misinterpreted because of the lack of control groups. The Gross Motor Function Measure Evolution Ratio (GMFMER) uses historical data from the Gross Motor Function Classification System curves and allows to re-analyze previous published studies which used the Gross Motor Function Measure by considering the natural expected evolution of the Gross Motor Function Measure. As the GMFMER is defined by the ratio between the recorded Gross Motor Function Measure score increase and the expected increase attributed to natural evolution during the duration of the study (natural evolution yields a GMFMER of 1), it becomes easy to assess and compare the efficacy of different treatments.

Objective: The objective of this study is to revisit studies done with different dosage of hyperbaric treatment and to compare the GMFMER measured in these studies with those assessing the effects of various recommended treatments in children with cerebral palsy.

Methods: PubMed Searches were conducted to included studies that used the Gross Motor Function Measure to evaluate the effect of physical therapy, selective dorsal rhizotomy, botulinum toxin injection, hippotherapy, stem cell, or hyperbaric treatment. The GMFMER were computed for each group of the included studies.

Results: Forty-four studies were included, counting 4 studies evaluating the effects of various dosage of hyperbaric treatment in children with cerebral palsy. Since some studies had several arms, the GMFMER has been computed for 69 groups. The average GMFMER for the groups receiving less than 2 h/week of physical therapy was 2.5 ± 1.8 whereas in context of very intensive physical therapy it increased to 10.3 ± 6.1. The GMFMER of stem cell, selective dorsal rhizotomy, hippotherapy, and botulinum toxin treatment was, 6.0 ± 5.9,

6.5 ± 2.0, 13.3 ± 0.6, and 5.0 ± 2.9, respectively. The GMFMER of the groups of children receiving hyperbaric treatment were 28.1 ± 13.0 for hyperbaric oxygen therapy and 29.8 ± 6.8 for hyperbaric air.

Conclusion: The analysis of the included studies with the GMFMER showed that hyperbaric treatment can result in progress of gross motor function more than other recognized treatments in children with cerebral palsy.

KEYWORDS

hyperbaric treatment, cerebral palsy, gross motor function, physical therapy, selective dorsal rhizotomy, hippotherapy, stem cell therapy, botulinum toxin injection

1 Introduction

Cerebral palsy (CP) is an umbrella term that describes a group of permanent neurological disorders caused by a brain defect or injury that occurred before, during birth or in the first months after birth. It is a non-progressive condition characterized by motor and muscle tone abnormalities and is the most prevalent motor disorder in children as it affects between 1.5 to 4 infants per thousand live births (1). Children with cerebral palsy have impaired muscle control and coordination, leading to problems with walking, balance, as well as fine motor skills (2). Depending on the brain area damaged, it can also affect speech, deglutition, cognition, vision and hearing. Currently, the cornerstone of treatments to improve motor function in children with CP includes rehabilitation therapies, braces, assistive devices, medications, and surgeries (3). These therapeutic approaches aim to help the children in achieving maximum potential in development, motor abilities, function and autonomy as well as preventing and treating secondary conditions such as musculoskeletal deformities.

State of evidence on the different approaches that aim to improve motor function in children with CP has been published and recently updated (3, 4). Among the reviewed therapies, several were reported as being effective and recommended to improve motor function in children with CP. These therapies included physical, pharmacological, and surgical approaches. However, over the past decades, new approaches have been developed with the purpose of improving the brain function and, thereby, the motor function. For some, despite promising results, the level of evidence concerning their efficiency seemed to be insufficient (3). Among those therapies, hyperbaric treatment (HBT) is a medical treatment that involves breathing various concentration of oxygen in a pressurized chamber (5, 6). It is used to treat various conditions, including decompression sickness, wounds that are difficult to heal, and carbon monoxide poisoning. HBT increases delivery of oxygen to the body, which can help to improve the function of damaged cells, reduce inflammation, and promote healing (7). In this review we will consider that HBOT is a HBT which provides pressurized oxygen (100% O2) and HBAT is a HBT which provides pressurized air (21% O2). One of the goals of HBT for cerebral palsy is to increase oxygen supply to the brain, which might improve neurological function (7). Indeed, breathing in a pressurized environment results in higher levels of oxygen dissolved in the blood plasma –breathing just air at 1.3 ATA results in an increase close to 50% of blood oxygen level (7) and thereby enhancing oxygen delivery to tissues throughout the body, including the brain. Moreover, studies have shown that stem cell mobilization is significantly increased following HBT. MacLaughlin et al. (8) reported that an exposition to hyperbaric air at 1.27 ATA generates up to a three-fold increase in circulating stems cells. Finally, HBT has been shown to up- or down-regulated the expression of thousands of genes; the largest clusters of upregulated genes were the anti-inflammatory genes and those that coded for growth and repair hormone, and the largest clusters of downregulated genes were the pro-inflammatory genes and apoptotic genes (9, 10). Together, these mechanisms could stimulate cerebral plasticity and lead to motor function improvement. Until now the evidence supporting the use of HBT for cerebral palsy has remained relatively limited, making it difficult to argue definitive conclusions about its efficacy. Indeed, the studies conducted so far have often been small-scaled or lacked the presence of a control or placebo group. The controversy on the efficacity of HBT in CP is still going on as some studies have wrongly considered mild hyperbaric pressures as a sham treatment for control groups (11, 12). These claims have been increasingly contested as many powerful healing mechanisms are activated even at very low pressure (8, 13–15).

In the last decades, the Gross Motor Function Measure (GMFM) (16), has been the most utilized standardized tool to evaluate gross motor function in children with CP (17, 18). The GMFM is an observational and reliable tool, easily applied by physiotherapists. To date, GMFM is considered as the best clinical standardized tool for measuring change in gross motor function over time in children with CP (19). During childhood, in a context of standard rehabilitation program as done in developed country, the GMFM score is expected to increase before reaching a plateau (20). Hence, to highlight the effect of treatment, the natural expected increase of the GMFM should be taken into account using a control group (21). Due to the heterogeneity of the CP population and ethical issue, controlled studies are hard to implement and most of them, including HBT studies, report GMFM score variation without comparison with a control group (4, 22). Uncontrolled studies generally determine the effects of a therapy based only on the GMFM variation without considering the expected progression of the GMFM due to natural development and standard rehabilitation (20), which depend on the child's initial age, motor function level, as well as the study duration. From this perspective, Marois et al. (21) created the Gross Motor Function Measure Evolution Ratio (GMFMER) which is the ratio between measured changes and the expected natural evolution (ENE) of the GMFM for a group of children with CP having the same age and disability level. The ENE is computed based on the reference gross motor function classification system (GMFCS) curves of Hanna et al. (20). It is then possible to compare this natural evolution with the GMFM gain observed during

the therapy and to compute the GMFMER. This is a novel and more comprehensive method that allows to interpret the results of treatments with higher levels of accuracy when a control group is not included in the study design. It also allows us to reanalyze previous published studies that were using the GMFM as an evaluation tool and to better estimate the effects of those interventions (21).

Many previous studies were misinterpreted because of the lack of comparison groups. In those situations, the GMFMER can provide a more accurate assessment of HBOT impact on motor function in children with CP. This paper aims to use the GMFMER to revisit studies done with different dosages of HBT and to compare these results to studies assessing the effects of recommended treatments for children with CP.

2 Materials and methods

Two searches were conducted by two members of the research team (PM, LB) on the electronic databases PubMed from January 1992 until October 2023. On one hand, using the key words "GMFM" AND "cerebral palsy" a systematic analysis was performed to identify studies which used the GMFM to evaluate the impact of standard treatments in children with CP. Studies reporting results from physical therapy (PT), selective dorsal rhizotomy (SDR), Botulinum treatment (BT), stem cell, and hippotherapy treatment were targeted because these treatments are recommended for children with CP (3). Study inclusions were based on the reading of the title, the abstract, and the full paper, as needed to respond to the inclusion criteria. In case of evaluators disagreement or doubt on criteria interpretations, discussion between evaluators was initiated to reach a final decision. All the studies which responded to the following inclusion criteria were included, (i.) English-language full paper, (ii.) evaluate the impact of a longitudinal treatment, (iii) report the average total score of the GMFM before treatment, (iv) report the GMFM increase or the GMFM total score after treatment, (v) report the time interval between evaluations, (vi) report the average age of the group, (vii) the average age of the group superior to 1 and inferior to 8 years old at the pre- and post-treatment evaluation, respectively, (viii) number of children in each group superior to nine, (ix) more than one study reported the effect of the treatment, and (x) group of children mostly presenting gross motor involvement either with quadriplegia or diplegia. Therefore, groups of children having unilateral CP were excluded because the GMFCS does not apply with the same reliability (23). The search strategy was limited to the one described above considering that the aim was to have a valuable representation about the effect of the most standard therapy in children with CP. On the other hand, using the key words "hyperbaric" AND "cerebral palsy," a second search was performed to identified all the studies which used the GMFM to evaluate the impact of HBT in children with CP. For this search the references list of each included studies was screened to ensure, as much as possible, the inclusion of all the eligible studies. Studies were eligible if they responded to the above-mentioned inclusion criteria and, (xi) receive HBT treatment, therefore the information needed to compute the GMFMER were available for each group of each included study.

The Expected Natural Evolution (ENE) were computed for each included group using the website application http://gmfmer.ca/ (21). The parameters required to compute the ENE were the mean age of the groups, the average GMFM score of the group at the start and after the treatment, as well as the time interval between the pre- and post-treatment evaluations. Then the GMFMER was calculated by dividing the GMFM variation recorded during the study by the ENE. As an example, a GMFMER of 3 would mean that the children receiving a treatment progressed, during the time of the treatment, 3 times more than what he was expected to, considering the natural improvement of the GMFM with standard therapy (20, 21). The GMFMER resulting from the same treatment were reported as mean ± standard deviation.

3 Results

The PubMed search using the key words "GMFM" AND "cerebral palsy" gave 691 results. Forty studies (59 groups) responded to the inclusion criteria. The number and the reasons for exclusion were detailed in the studies flowchart (Figure 1). Among the included studies, 24 groups were involved in PT treatment. The intensity of the treatment was characterized based on the time dedicated to therapy per week. Less than 2 h (9 groups), between 2 and 5 h (7 groups), and more than 5 h of treatment (8 groups) were, respectively, defined as common therapy, intensive therapy and very intensive therapy. Groups which were involved in a PT program defined as "usual PT" or "usual care" were pooled with common PT groups. The PubMed search using the key word "hyperbaric" AND "cerebral palsy" gave 54 results. After references screening, 4 studies responded to the inclusion criteria. The number and the reasons for exclusion were also detailed in the studies flowchart (Figure 1). Among the included, 5 groups were treated with HBOT (1.5 or 1.75ATA), 2 groups with HBAT (1.3 ATA), and 1 group with HBT (1.3ATA and 14% O2).

GMFMER of the common PT groups were between 1.1 and 5.7 with a mean of 2.5 ± 1.8. The use of the GMFMER showed that children involved in intense PT (>2 h) improved between 1.8 and 8.6 times more than expected with the natural evolution. Groups of children receiving intensive PT had a GMFMER increase of 4.4 ± 2.7 and children receiving more than 5 h/week of PT had a mean GMFMER of 10.3 ± 6.1 (see Table 1). Rhizotomy produced improvements of GMFMER between 2.4 and 9.5 with an average of 6.5 ± 2.0 for periods up to 12 months. Stem cells treatments resulted in GMFMER between 2.1 and 17.6 with a mean of 6.0 ± 5.9. Mean GMFMER of hippotherapy group ($n = 2$) and BT groups ($n = 8$) were 13.3 ± 0.6 and 5.0 ± 2.9, respectively (see Table 2).

Finally, 7 groups from 4 studies reporting the effect of HBOT and HBAT in children with CP were included (see Table 3). The range of the GMFMER was between 16.4 and 47 with a mean of 28.6 ± 11.0. HBT groups were distributed into two subgroups: one composed by the groups ($n = 5$) treated with HBOT at pressures between 1.5 and 1.75 ATA, as the second ($n = 2$) received HBAT at 1.3 ATA. The mean GMFMER of the HBOT and HBAT groups were very similar, 28.1 ± 13.0 and 29.8 ± 6.8, respectively (see Figure 2). Lacey's "control" group (11) which received a very unusual HBT treatment at 1.5 ATA with just 14% O2, was analyzed separately and had a GMFMER of 8.5.

4 Discussion

The present study evaluating the effects of different treatments on children with CP with the GMFMER report original results to estimate

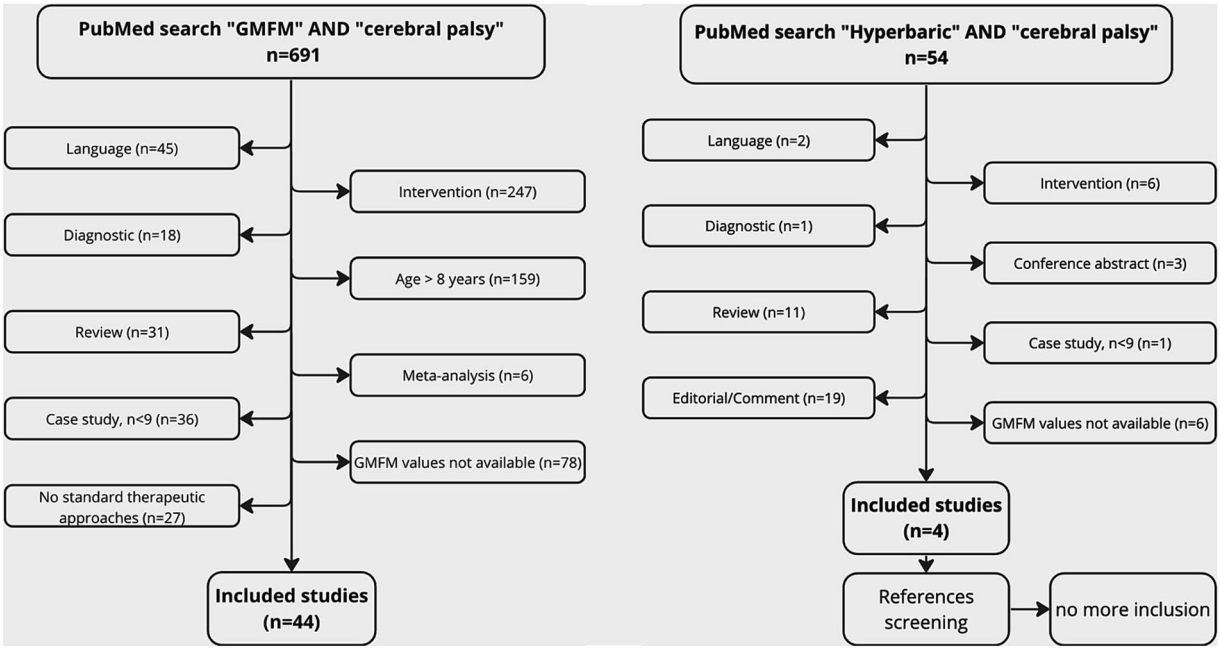

FIGURE 1
Flow chart of included studies.

the efficacy of recognized or commonly used experimental therapies in children with CP. In total, the GMFMER were calculated for 59 groups. As far as the authors know, previous analysis only considered GMFM improvements without taking into account natural improvement, making it harder to understand the true significance of the measured changes. The GMFMER was established using the GMFCS curves, which were produced using thousands of data points coming from children receiving standard therapy approximately once a week in Ontario, Canada (20). A GMFMER close to 1 is expected in groups of children receiving regular therapy equivalent to standard therapy in Ontario (21). For decades, intensive PT was considered as a powerful method to improve the gross motor skills in children with CP, but the lack of comparison with control groups in many studies implemented with this treatment was often leading to results that were difficult to interpret. Results of the present study showed that PT produce better gains when done more frequently. The GMFMER confirms that intensive PT yields better results, with an average GMFMER of 10.3 obtained for children treated 5 h per week or more (25, 33–38). There is no doubt that SDR is very efficient in decreasing spasticity in children with CP (64) but the true effects on motor function were also difficult to interpret. The results of the present study confirm that this surgery can produce important gains in gross motor function with a mean GMFMER of 6.5 (31, 34, 38, 46–53). In this case the GMFMER value demonstrate a particularly beneficial effect of the treatment, because it applies on a long period, twelve months in most studies (64). Stem cells treatment seems also promising but, as reported with the GMFMER, results are variable in children with CP with GMFMER range between 2.1 and 17.6 (mean, 6.0 ± 5.9) (24, 42, 60, 61). Some studies have reported improvements in motor function (42, 61), while others have shown limited effects with GMFMER values equivalent to those of regular PT (24, 60). Outcomes variability could be due to factors such as cell type, dosage, route of administration, patient age, and severity of cerebral palsy. Since the protocol for this type of treatment can vary tremendously, further analysis would be required to compare the outcomes of the different approaches.

The most important GMFMER value are obtained with HBT. The calculated GMFMER means that children improved their GMFM score on average 28.6 times more than what is naturally expected with standard therapy and 3 times more than with very intensive PT alone (43, 62, 63). The rate of increase of the GMFM score resulting from HBT is also 4 times more than with SDR treatment. Our analysis highlights that the children treated either with HBOT (1.5–1.75 ATA) or with HBAT (1.3ATA) have a very similar progress rate. Furthermore, HBOT and HBAT not only produces impressive gains in gross motor function but studies have also shown significant improvement in fine motor skills, speech, attention and memory (43, 62). The computed GMFMER clearly demonstrates the important change in gross motor skills that HBOT and HBAT can bring to children with CP. The results of the present study corroborates former studies done with people with other neurological conditions including stroke (65), brain trauma (66), autism (67, 68). These studies have also reported significant clinical results, which suggest the potential of this treatment in many types of brain dysfunction. While those results show positive impact on CP and other neurological conditions, these are still to this day not recognized indications for HBT. The most recent meta-analysis on HBT and CP, by Laureau et al. (12), concluded on its inefficacity. In our opinion this analysis was flawed in a multitude of ways. In their analysis, Laureau et al. (12) arrived at non favorable conclusion regarding HBT because of errors in their analysis and interpretation of the included randomized control trials. Indeed, the study published by Collet et al. (62) must be considered as a trial comparing two different dosage of HBT, not as a study comparing HBT to a sham or placebo treatment, as considered by Laureau et al. (12). More specifically, one group was treated with HBOT and the other with HBAT. In their review, Laureau et al. (12) considered

TABLE 1 Physical therapy in children with CP, 24 groups, $n = 560$.

References		Treatments	n	Age (months)	Treatment duration (months)	GMFM initial (%)	GMFM final (%)	GMFM gain	ENE	GMFMER
Sun et al. 2017	(24)	Common PT	31	37.2	12.0	52.0	58.9	6.9	4.4	1.6
Sel et al. 2023	(25)	Common PT	20	56.4	5.3	42.1	42.6	0.5	0.5	1.1
Stark et al. 2016	(26)	Common PT	12	19.4	3.2	32.2	35.5	3.3	2.8	1.2
Kwon et al. 2015	(27)	Common PT	46	70.8	2.0	61.4	61.8	0.4	0.2	2.1
Kwon et al. 2011	(28)	Common PT	16	76.8	2.0	69.8	70.1	0.3	0.2	1.4
Boyd et al. 2001	(29)	Common PT	20	39.0	12.0	40.4	43.2	2.8	2.5	1.1
Trahan et Malouin 1999	(30)	Common PT	24	48.4	8.0	42.1	48.3	6.2	1.1	5.7
Steinbok et al. 1997	(31)	Common PT	69	87.6	12.0	69.0	70.9	1.9	0.6	3.1
Tsorlakis et al. 2004	(32)	Common PT	17	85.2	4.0	65.9	67.1	1.2	0.2	5.2
			Mean	57.9	6.7	52.9	55.4	2.6	1.4	2.5
			SD	23.7	4.4	14.2	13.3	2.5	1.5	1.8
Steinbok, Reiner, et Kestle 1997	(33)	Intensive PT	14	47.0	9.0	62.7	67.8	5.1	2.8	1.8
Wright et al. 1998	(34)	Intensive PT	12	58.3	12.0	56.5	60.9	4.4	1.5	3.0
K. H. Lee et al. 2017	(35)	Intensive PT	24	68.3	9.0	55.3	58.5	3.2	0.7	4.8
Knox et Evans 2002	(36)	Intensive PT	15	88.0	18.0	54.7	55.6	0.9	0.1	6.3
S. H. Lee et al. 2015	(37)	Intensive PT	20	27.6	3.0	32.1	34.4	2.3	1.3	1.8
Sel et al. 2023	(25)	Intensive PT	23	55.2	5.3	40.2	44.2	4.1	0.5	8.6
McLaughlin et al. 1998	(38)	Intensive PT	17	86.4	12.0	71.3	75.5	4.2	0.7	6.0
			Mean	61.5	9.8	53.3	56.7	3.4	1.1	4.4
			SD	21.5	4.9	13.2	13.9	1.4	0.9	2.7
Yi et al. 2013	(39)	Very intensive PT	45	41.0	1.7	48.3	55.5	7.2	0.6	13.0
Polovina et al. 2010	(40)	Very intensive PT	12	17.0	50.5	15.2	52.4	36.8	7.3	5.0
Tsorlakis et al. 2004	(32)	Very intensive PT	17	90.0	4.0	62.2	64.5	2.3	0.2	14.4
Chaturvedi et al. 2013	(41)	Very intensive PT	18	52.8	6.0	44.0	50.0	6.0	0.7	8.5
Huang et al. 2018	(42)	Very intensive PT	27	90.0	24.0	85.0	89.8	4.8	1.8	2.8
Mukherjee et al. 2014	(43)	Very intensive PT	20	42.0	6.0	29.6	32.4	2.8	0.7	3.8
Christy, Chapman, et Murphy 2012	(44)	Very intensive PT	17	91.2	3.0	61.8	64.0	2.2	0.1	18.0
M. Lee et al. 2015	(45)	Very intensive PT	24	28.3	1.0	24.5	29.6	5.1	0.3	17.1
			Mean	56.5	12.0	46.3	54.7	8.4	1.5	10.3
			SD	29.9	17.2	23.1	19.2	11.6	2.4	6.1

ENE, expected natural evolution; GMFM, gross motor function measure; GMFMER, gross motor function measure evolution ratio; PT, physical therapy.

TABLE 2 Commonly used therapy in children with CP, 27 groups, $n = 986$.

References		Treatments	*n*	Age (months)	Treatment duration (months)	GMFM initial (%)	GMFM final (%)	Gain GMFM	ENE	GMFMER
Pennington et al. 2020	(46)	SDR	137	78.5	12.0	59.0	63.6	4.6	0.6	7.5
Wright et al. 1998	(34)	SDR	12	57.8	12.0	51.9	64.0	12.1	1.3	9.5
Law et al. 1997	(47)	SDR	18	51.6	12.0	48.2	57.8	9.6	1.6	6.2
van Schie et al. 2011	(48)	SDR	24	79.0	12.0	56.6	60.9	4.3	0.5	8.0
van Schie et al. 2005	(49)	SDR	9	65.3	12.0	62.8	71.5	8.7	1.3	8.7
Sargut et al. 2021	(50)	SDR	77	72.0	24.0	70.0	77.0	7.0	2.9	2.4
Summers et al. 2019	(51)	SDR	137	72.0	24.0	59.0	66.2	7.2	1.3	5.3
Zhan et al. 2020	(52)	SDR	86	74.4	19.9	53.0	59.1	6.1	0.8	7.4
McLaughlin et al. 1998	(38)	SDR	21	76.8	12.0	70.3	75.2	4.9	1.0	4.9
Nordmark, Jarnlo, et Hägglund 2000	(53)	SDR	18	51.6	12.0	48.2	57.8	9.6	1.6	6.2
Steinbok et al. 1997	(31)	SDR	14	50.0	9.0	60.7	72.0	11.3	2.2	5.2
			Mean	66.3	14.6	58.2	65.9	7.8	1.4	6.5
			SD	11.5	5.3	7.6	7.0	2.7	0.7	2.0
				66.3	14.6	58.2	65.9	7.8	1.4	6.5
Colovic et al. 2012	(54)	BT	16	49.0	6.0	67.8	70.9	3.1	2.1	1.5
Chaturvedi et al. 2013	(41)	PT + BT	18	51.6	6.0	52.0	59.0	7.0	1.03	6.8
Linder et al. 2001	(55)	BT	25	60.0	12.0	54.9	71.1	6.2	1.3	4.9
Moore et al. 2008	(56)	BT	30	63.6	24.0	66.5	71.8	5.3	2.6	2.0
Chang et al. 2017	(57)	BT	71	64.8	5.3	70.5	73.7	3.2	0.9	3.5
Kim, Rha, et Park 2020	(58)	BT	29	85.2	3.5	78.6	81.2	3.5	0.3	10.6
Matsuda et al. 2018	(59)	BT	9	75.6	2.8	62.3	63.3	1.0	0.2	4.6
Polovina et al. 2010	(40)	PT + BT	12	23.0	56.0	20.8	58.9	38.1	6.0	6.3
			Mean	59.1	14.4	59.2	68.7	8.4	1.9	5.0
			SD	18.8	18.2	17.7	7.8	12.1	2.0	2.9
Kwon et al. 2015	(27)	Hippotherapy	45	68.4	2.0	60.8	63.5	2.7	0.2	12.9
Kwon et al. 2011	(28)	Hippotherapy	16	73.2	2.0	70.4	73.7	3.3	0.2	13.8
			Mean	70.8	2.0	65.6	68.6	3.0	0.2	13.3
			SD	3.4	0.0	6.8	7.2	0.4	0.0	0.6
Sun et al. 2022	(60)	Stem cell	23	45.6	12.0	50.3	58.8	8.6	2.4	3.6
Sun et al. 2022	(60)	Stem cell	25	43.2	12.0	48.1	54.7	6.6	2.6	2.6
Sun et al. 2022	(60)	Stem cell	20	43.2	12.0	49.0	58.0	9.0	2.7	3.4
Yousif et al. 2023	(61)	Stem cell	35	51.6	3.0	29.0	32.7	3.7	0.2	17.6
Sun et al. 2017	(24)	Stem cell	32	38.4	12.0	48.9	56.4	7.5	3.6	2.1
Huang et al. 2018	(42)	Stem cell	27	87.6	24.0	85.0	97.7	12.7	1.9	6.6
			Mean	51.6	12.5	51.7	59.7	8.0	2.2	6.0
			SD	18.2	6.7	18.2	21.0	3.0	1.1	5.9

BT, botulinum toxin, ENE, expected natural evolution; GMFM, gross motor function measure; GMFMER, gross motor function measure evolution ratio; PT, physical therapy; SDR, selective dorsal rhizotomy.

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TABLE 3 HBOT, HBAT and HBT in children with CP, 8 groups, *n* = 316.

References		Treatments	*n*	Age (months)	Treatment duration (months)	GMFM initial (%)	GMFM final (%)	Gain GMFM	ENE	GMFMER
Collet et al. 2001	(62)	HBOT-1.75ATA	57	86.4	2.0	57.3	60.2	2.9	0.1	36.3
Mukherjee et al. 2014	(43)	HBOT-1.75ATA	58	51.6	6.0	32.5	42.1	9.6	0.5	20.9
Mukherjee et al. 2014	(43)	HBOT-1.5ATA	32	51.6	6.0	34.3	42.5	8.2	0.5	16.4
Lacey, Stolfi, et Pilati 2012	(11)	HBOT-1.5ATA	25	75.6	2.0	39.5	40.7	1.2	0.1	20.0
Montgomery et al. 1999	(63)	HBOT-1.75ATA	25	67.2	1.0	56.9	61.6	4.7	0.1	47.0
			Mean	66.5	3.4	44.1	49.4	5.3	0.2	28.1
			SD	15.2	2.4	12.1	10.5	3.5	0.2	13.0
Collet et al. 2001	(62)	HBAT-1.3ATA	54	86.4	2.0	66.3	69.3	3.0	0.1	25.0
Mukherjee et al. 2014	(43)	HBAT-1.3ATA	40	58.8	6.0	29.6	38.6	9.0	0.3	34.6
			Mean	72.6	4.0	48.0	54.0	6.0	0.2	29.8
			SD	19.5	2.8	26.0	21.7	4.2	0.1	6.8
Lacey, Stolfi, et Pilati 2012	(11)	HBT-1.5ATA + 14%O2	25	62.5	2.0	40.7	41.8	1.1	0.1	8.5

ENE, expected natural evolution; GMFM, gross motor function measure; GMFMER, gross motor function measure evolution ratio; HBAT, hyperbaric air treatment; HBOT, hyperbaric oxygen treatment; HBT, hyperbaric treatment.

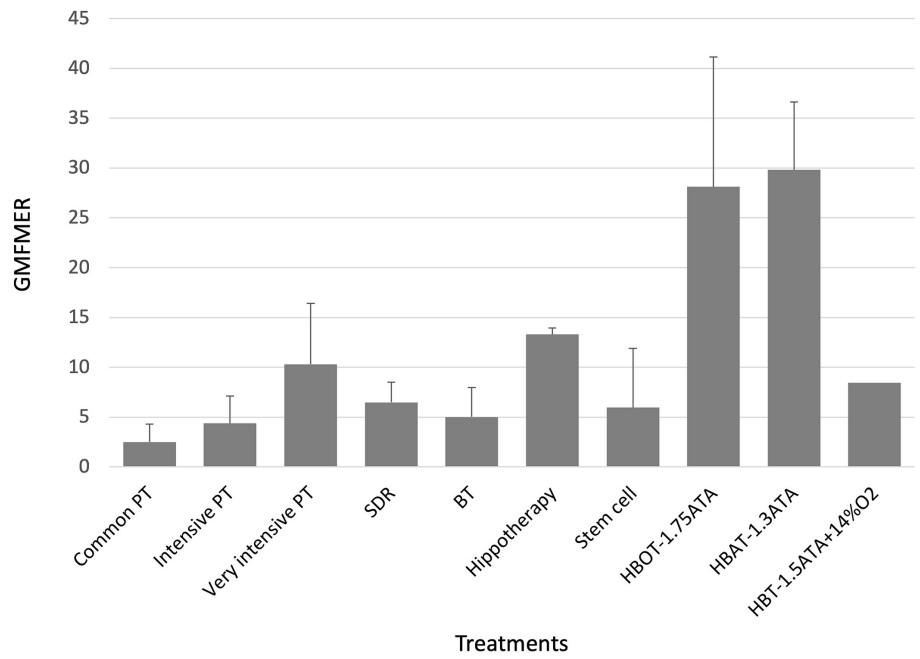

FIGURE 2
GMFMER for physical therapy, selective dorsal rhizotomy, Botulinum toxin; HBOT, HBAT, HBT, hippotherapy, stem cell therapy. BT, botulinum toxin; GMFMER, gross motor function measure evolution ratio; HBAT, hyperbaric air treatment; HBOT, hyperbaric oxygen treatment; HBT, hyperbaric treatment; PT, physical therapy; SDR, selective dorsal rhizotomy.

the group receiving HBAT as a control and this false assumption completely changes their interpretation of the study. Regarding the physiological effect of even low pressured air and the value of the GMFMER reported in the present study, this group should definitively not be considered as a control one to assess HBOT effect. In fact, both groups in this study had a significant improvement of their gross motor function, as well as their speech, attention, memory, and functional skills (62, 69). These points were rightly noted in the Lancet's editorial that suggested reinterpretation of Collet et al.'s study (13, 62). Moreover, in 2003, the US Agency for Healthcare Research and Quality (AHRQ) concluded that in Collet's study "The possibility that pressurized room air had a beneficial effect on the motor function should be considered the leading explanation" when analyzing this study (62, 70).

In addition, Laureau et al. (12) did not use the GMFMER to analyze the studies reviewed in their meta-analysis, however they stated that "the GMFMER should be used as the outcome measure for motor function in children with CP, rather than the GMFM, especially for interventions performed over a long period, like HBT." If they had computed the GMFMER value of the two treatment groups in Collet's study, they would have found that they were 36.3 and 25.0 for HBOT and HBAT, respectively. These numbers invalidate the possibility that these changes could be attributed to a participation effect or children natural evolution. It is even more confusing that Laureau et al. (12) wrongly classified HBAT (1.3 ATA) as a control group, while they have classified the same treatment as hyperbaric in their analysis of Mukherjee et al.'s study (12, 43) – it certainly cannot be both. A second major error was made in their analysis of Lacey's study (11), where once again, a group receiving HBT at 1.5 ATA but breathing only 14% oxygen was used as control group. This lower level of oxygen paired with the 1.5 ATA pressurization has been attempted to replicate the levels of oxygen perfusion that would normally be observed under ambient air. However, pressurization alone induce many physiological changes regardless of oxygenation, and it has been shown repeatedly that many powerful healing mechanisms can be activated even with a limited pressure increase (5, 8, 14, 15). For this reason, it is inaccurate to consider any group receiving HBT as a control, regardless of their levels of oxygenation (8, 71–73). Another critical point regarding the Lacey et al.'s study (11) is the fact that they arrived at a negative conclusion regarding HBT whereas they did not complete their study, which was initially planned for 8 weeks (40 treatments). Surprisingly, they also decided to excluded children who had evidence of neonatal hypoxic–ischemic encephalopathy. Based on an interim analysis the trial was stopped after 2 months because the GMFM increase was inferior to 5 in the HBOT groups. This threshold was arbitrarily chosen and did not correspond to a realistic increase of the GMFM score for the groups of children included in this study. Indeed, by using the average starting age, GMFM score of the groups studied, and computing the expected natural evolution during those 2 months, a GMFM increase of 5 over the course of the study corresponds to a GMFMER of 83.3. No treatment has ever shown such drastic improvements in children with CP. It is very questionable to look at absolute GMFM increase to assess the efficacy of a treatment as this increase is highly dependent on the starting age and duration of the study, which is the exact reason the metric of GMFMER was conceived. With Lacey's data, i.e., GMFM increase of 1.2 for HBOT group and 1.1 for the HBT ("control") group, the GMFMER values were 20 and 8.5, respectively. The result of this second group is quite impressive, considering that this group was breathing a mixture of gases with a concentration of only 14% oxygen but with a pressurization of 1.5 ATA (11). It certainly yields to some reflection and suggest that the positive changes obtained with HBT or HBAT could be possibly more related to the pressure (even mild) than the oxygen dose. Finally, Lacey's study cannot be considered as a proof against HBT in CP. Comments expressing deep concerns about the interpretation and conclusion of this study were already published as a Letter (74).

Studies form Fratantonio et al. (75) or Balestra et al. (76) involving humans give reflection and a better understanding of physiological phenomena to pressure exposure and increase in partial pressure of oxygen. The activation and expression of genes involved in response to ambient air pressure variation could be explained by the "normobaric oxygen paradox." Indeed, they highlight important adaptive responses triggering signaling cascades leading to better known expressions of antioxidant systems such as transcriptional activation of Hypoxia inducible factors (HIF-1α) and microparticles expressing cell-specific proteins leading to DNA repair in various tissues. Mac Laughlin et al. (8) recently published a paper evaluating the effects of HBAT (at 1.27 ATA) on stem cells mobilization. They showed that endogenously mobilized stem and progenitor cells (SPCs) (CD45dim/CD34+/CD133-) were increased by nearly two-fold following 9 exposures ($p = 0.02$) increasing to three-fold 72-h post completion of the final (10th) exposure ($p = 0.008$) confirming durability. Authors concluded that "stem cells (SPCs) are mobilized, and cytokines are modulated by hyperbaric air. HBA likely is a therapeutic treatment. Previously published research using HBA placebos should be re-evaluated to reflect a dose treatment finding rather than finding a placebo effect."

5 Study limitation

There are several limitations related to this study that should be considered in interpreting the results. First, the studies that we analyzed had all the data needed for the calculation of the GMFMER. They all included GMFM measurements. However, some of the older studies used the GMFM-88 instead of GMFM-66. While these two measures are considered to yield similar results (77), the GMFMER was created using the GMFCS curves developed using data evaluating children with GMFM-66. For this reason, it is possible that GMFMER results computed for therapies using GMFM-88 might vary slightly. Second, the GMFMER cannot be used in all the studies done in CP as other types of pertinent evaluations or scores are also regularly used. Third, there was a wide variability in the qualities of the studies as some were randomized or controlled and others were pilot or observational studies. There was also a wide range in the treatment's protocol of some of the evaluated therapies, especially with stem cells or PT. Fourth, some of the studies were evaluating the effects of a specific therapy that was associated with a secondary treatment. The best example are the studies on SDR in CP as the children also received regular or intensive PT during the whole follow-up period. Consequently, we have to interpret our data and our analysis with some caution and recognize that in some cases it is the combined effects of therapies that produced improvements. Finally, we also have to bring nuances in the interpretation of the GMFMER results as it evaluates the progress obtained by various therapies over the time of duration of the therapy; in certain cases, that increase is maintained over a period of 6 or more months, as seen with SDR or HBT, while the effect can be less prolonged with other treatments.

6 Conclusion

The present study compared the effects of HBT with those of other currently used therapy in CP. The use of GMFMER clearly demonstrates that both HBOT and HBAT lead to gross motor function improvements. Based on the GMFMER these improvements are more important than with any other therapy for children with CP. There is no scientific argument that could bring into question the validity of HBT as a treatment for CP. Our data shows that even HBAT at 1.3 ATA can produce GMFM gains much greater than with standard care and produces the same amount of motor improvements as those obtained at higher pressures. It is therefore scientifically fallacious to use such a treatment for control groups when designing or reviewing studies.

HBAT is a very simple treatment when it is done at these low pressures and can be given in portable softshelled chambers. Considering the benefits of HBAT on gross motor function in children with CP, the use of HBAT combined with recognized therapy for all children with CP should be recommended. Well-designed multicenter trials are still welcomed to determine with more precision the best dosages, frequency of administration and indications for different types and etiologies of CP and possibly other neurological conditions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

PM: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. GL: Formal analysis, Investigation, Writing – review & editing. MM: Formal analysis, Investigation, Methodology, Writing – review & editing. LB: Formal analysis, Investigation, Writing – review & editing, Conceptualization, Methodology, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Hyperbaric oxygen therapy for thalamic pain syndrome: case report

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Thalamic pain syndrome is a distressing type of central post-stroke pain (CPSP) that occurs in up to 10% of cases following a cerebrovascular accident, typically with a delayed onset of signs and symptoms, and is often chronic or even life-long. Thalamic pain syndrome, as is the case for other CPSPs, is difficult to treat, and the response is typically moderate at best. Central pain also occurs after vascular insults in parts of the CNS other than the thalamus. Only a few patients present with the classic "Dejerine and Roussy syndrome," so the term CPSP is preferred for describing neuropathic pain after stroke. There are no pathognomonic features of this syndrome. The thalamus probably has a substantial role in some patients with central pain, either as a pain generator or by abnormal processing of ascending input. Long-term post-stroke pain disorders can reduce the quality of life, affect mood, sleep, and social functioning, and can lead to suicide. Hemi-body pain is common in patients with thalamic lesions. Hyperbaric oxygen has known physiologic and pharmacologic effects with documented benefits in brain-related hemorrhages, acute and chronic stroke, traumatic brain injury, mild cognitive impairment, neurodegenerative diseases, and neuroprotection, but has never been reported as a treatment for thalamic pain syndrome. A 55-year-old man with a history of migraines suffered a right thalamic lacunar infarction following a brain angiogram to investigate a suspected AVM found on prior imaging that resulted in immediate left-sided weakness and numbness, evolving to severe chronic pain and subsequent stiffness. Diagnosed with thalamic pain syndrome, multiple pharmacologic therapies provided only partial relief for a year after the stroke. The patient's symptoms resolved and quality of life markedly improved with hyperbaric oxygen therapy, as assessed by multiple validated questionnaires, thus it may be a treatment option for thalamic pain syndrome.

KEYWORDS

thalamic pain syndrome, hyperbaric oxygen, case report, central post-stroke pain, post-stroke thalamic pain

Introduction

This is the first known case report of the use of hyperbaric oxygen (HBO2) for the treatment of thalamic pain syndrome, also known as Dejerine–Roussy syndrome, a type of central post-stroke pain (CPSP), also known as post-stroke thalamic pain (PS-TP) (1).

Thalamic pain syndrome is a debilitating disease process that has limited treatment options.

A pure sensory stroke is a well-defined clinical entity with predominant hemisensory symptoms without other major neurological signs (2). The prognosis is typically poor. The

character and severity of the pain will be persistent and often unchanging.

The prevalence of thalamic pain syndrome following a stroke is up to 10% of cases, and the onset of symptoms is often delayed, with the patient not experiencing significant pain until months or years after the stroke.

There are multiple reports of treatments for thalamic pain syndrome, including tricyclic antidepressants, anticonvulsants and opioid analgesics, intravenous morphine, lidocaine, and propofol (3), naloxone infusions (4), and Cilostazol (5). Proposed non-pharmacological treatments include neurostimulation (motor cortex, deep brain, and transcranial magnetic stimulation) for treatment-resistant cases of CPSP (3, 5), mirror therapy (6), ipsilateral stellate ganglion block (7), botulinum neurotoxin A injection (8), precentral gyrus stimulation, caloric vestibular stimulation, transcranial direct current stimulation, and bee venom acupuncture point injection (9).

HBO2 has been reported to be an effective treatment for multiple brain-related diagnoses, including TBI, acute stroke, neurodegenerative disease, and cognitive impairment. Proposed mechanisms of action include amelioration of oxidative stress, activating endogenous antioxidant activity, modulation of neuroinflammation, inhibiting apoptosis, stimulating pathways of neuroprotection, and modulating cerebral blood flow and brain metabolism (10).

In a randomized, prospective trial, the authors conclude that HBO2 during the degenerative (or acute post-stroke) stage could increase post-injury damage but that elevated oxygen during the regenerative stage would supply the energy needed for the innate brain repair processes. The study convincingly demonstrates that HBO2 can induce significant neurological improvement and that neuroplasticity can be operatively activated in chronic late-stage stroke patients long after the acute brain insult (11).

Case description

This is the case of a 55-year-old man with thalamic pain syndrome. His past medical history includes asthma, CAD, diabetes type 2, hyperlipidemia, hypertension, and migraines. His past surgeries include gastric bypass, lumbar, and cervical fusions. A twin brother died in 2016 with end-stage renal disease and multiple strokes.

An MRI performed to evaluate migraines was significant for a focal prominence of the anterior portion of the inferior sagittal sinus, thought to represent a venous varix or focal tortuosity; it did not show arteriovenous shunting. The patient was referred to interventional radiology for further evaluation, with a cerebral angiogram conducted on 30 December 2021. The patient suffered an acute post-procedural thromboembolic right thalamic lacunar infarction, resulting in left-sided weakness and numbness including grip and foot drop. The patient was hospitalized and treated appropriately for an acute thromboembolic stroke with IV tenecteplase (TNK). Echo was normal without PFO, LDL 73, and A1c 5.8. The patient was placed on aspirin 325. A brain CT on 3 January 22 showed a 1 cm right thalamus lesion consistent with an acute infarct. Subsequent MRIs showed multifocal posterior circulation strokes, including the right MCA territory, notably the right thalamus (Figure 1).

The patient's grip weakness and foot drop resolved completely in the subacute post-stroke stage, but numbness persisted. The patient developed severe left-sided burning hyperalgesia with an aftersensation described as a painful left hemi-body stiffness, which worsened 9 months post-cerebral vascular accident (post-CVA). In the ensuing year after the CVA, the patient experienced chronic, recurrent, frequent symptoms of intense acute left anterior chest burning pain that variably included the left face, followed predictably by a left hemi-body tightness/stiffness sensation that initially occurred 8–10 times/month, lasting an average of 2–3 days, rated 10/10 on the pain scale. Pain was paroxysmal burning or tingling, sometimes from the left ear distally.

The patient was evaluated in the ED over 10 times during that year for acute episodes and diagnosed with thalamic pain syndrome. Acute coronary syndrome was considered and ruled out.

Medical therapy included an anticonvulsant (gabapentin 300–600 gm t.i.d.), that variably provided partial relief approximately 1 h after administration, and a serotonin–noradrenaline reuptake inhibitor (SNRI, duloxetine 30–60 mg daily). The patient was initially treated with muscle relaxants, Methocarbamol, then baclofen (dose), and later cyclobenzaprine 10 mg t.i.d. PRN, all of which were subsequently discontinued due to marginal reported benefit. Pain medications included Norco 2/325 q6h PRN, Ibuprofen 600 mg q.i.d., PRN, and Tylenol. The patient reported symptomatic improvement with two short courses of Valium given during emergency room visits but was never prescribed for maintenance therapy.

The patient was managed by neurology as an inpatient, but not evaluated by outpatient neurology until 1 year post-CVA (Tables 1, 2).

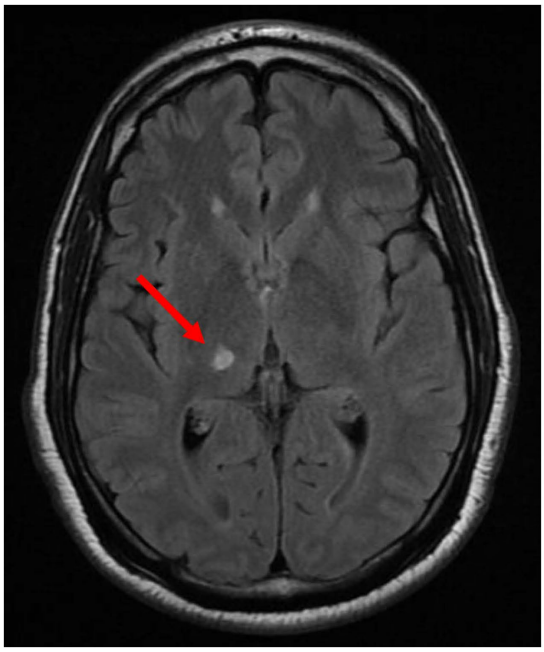

FIGURE 1
MRI January 2022 showing right thalamic lacunar infarction.

TABLE 1 Outcome measurements and assessments.

	Initial/Baseline (completed retrospectively for January 2022)	After 57 HBO2 treatments (14 August 23)	After 71 HBO2 treatments (5 September 23)	After 80 HBO2 treatments (20 September 23)	Rating Scale
Pain Disability Index	39 Completely disabled	28 Severe disability	5 Mild disability	10 Mild disability	Increasing severity (0–60)
McGill Pain Questionnaire (SF-MPQ-2)	172 "Horrible"	89	60 "Mild"	6 No pain	Increasing severity (0–220)
Visual Analog Scale of Pain (DVPRS)	9.25	(No data)	2.75	1	Increasing Severity (0–10)
Beck Anxiety Inventory	49 Potentially concerning level of anxiety	3 Low anxiety	5 Low anxiety	4 Low anxiety	Low (0–21) Moderate (22–35) Potentially concerning (>/= 36)
Beck Depression Inventory	19 Borderline clinical depression	3 Normal	6 Normal	8 Normal	1–10 normal ups and downs 11–16 mild mood disturbance 17–20 borderline clinical depression 21–30 moderate depression 31–40 severe depression >40 extreme depression
EuroQol (Eq-5D-5L)	11/30	6/95	6/90	6/96	Level sum score (LSS)/VAS LSS: 0–5 no problems, 6–10 slight, 11–15 moderate, 16–20 severe, 21–25 extreme problems VAS: worst to best health (0–100)
Columbia Suicide Severity Rating Scale	0	0	0	0	Any score greater than 0 is important and may indicate the need for mental health intervention
Montgomery-Asberg Depression Rating Scale	3	5	No data	6	0–6 normal, symptoms absent 7–19 mild depression 20–34 moderate depression 35–60 severe depression

These validated tools are widely used and were selected from the outcome measurements suggested by Plow, et al. in a TPS RCT proposal (Trials. 2013. 14[241]).

Diagnostic assessment

Criteria for the diagnosis of CPSP include pain in an area of the body corresponding to the CNS lesion, a history suggestive of stroke and pain onset at or after stroke onset, confirmation of the CNS lesion by imaging, and other causes of pain excluded or considered highly unlikely (3).

Diagnosis of PS-TP based on contralateral burning pain and spasticity after an imaging-demonstrated right thalamic ischemic stroke

TABLE 2 Timeline with relevant data from the episode of care.

29 Jun 2021 MRV Brain w/o contrast	4 mm venous outpouching from the inferior sagittal sinus may represent small arteriovenous fistula or arteriovenous malformation as it appears to have a component of arterial flow. Definitive evaluation of this structure can be obtained with catheter angiography.
30 December 2021 Cerebral angiogram	Acute CVA after femoral catheter removal complicated by post-procedural thromboembolic stroke with residual left-sided paresthesias and weakness.
4 January 2022	Admitted for suspected ACS, history of recurrent typical angina with undetectable cardiac troponin I levels on admission. Cardiac catheterization showed mild, non-obstructive coronary artery disease with 10–20% mid RCA stenosis, and 20–30% stenosis in the mid-distal RCA. Medical therapy was optimized.
10 January 2022	Seen in the ED for left-sided chest pain. CT head, MRI brain.
3 January 2023	Initial HBO2 consult, pain 10/10.
30 January 2023 (HBO2 #16)	Pain 4–6/10 -decreased duration of daily episodes. Decreased intensity, less stiffness.
10 February 2023 (#22)	Decreased severity and duration of flares.
16 February 2023	Continue gabapentin, duloxetine, stop Tylenol.
28 February–10 April 2023 (#33)	Break for a trip, 41 days.
10 April 2023	Return from trip, some recurrent episodes but not as severe as a baseline.
13 April 2023 (#36)	No left thorax burning paresthesia since December 2022, continued left-sided stiffness, less intense.
2 May 2023 (#47)	1, 2 May – worse stiffness, some relief with Tylenol, shorter duration than baseline.
15 May–14 Aug 2023 (#56)	Trip, no HBO2 for 90 days.
14 August 2023 (#57)	Return from trip, incremental loss of prior benefit.
18 September 2023 (#80)	Last planned HBO2 treatment, continued symptomatic improvement.
18 September–17 October 2023	Trip of 28 days.
17 October 2023	Decision to resume HBO2 with a plan for 20 treatments or to the plateau or resolution of symptoms.
15 November 2023 (#100)	Last HBO2 treatment, complete symptoms resolution, continues gabapentin, and duloxetine.

ED: 3–4, 10–11, 15, 18, 25 January 22, 7 September 2022, 19 December 2022, 1 November 2023.
Admit: 4, 11, 16, 25–28 January 2022, 19–20 December 2022.

lesion that occurred at the time of a cerebral angiogram performed for a migraine workup, with associated showering of emboli.

One year post-CVA, the patient's symptoms remained unabated, with partial, temporary relief from gabapentin 300–600 mg TID, duloxetine 60 mg daily, a muscle relaxant (baclofen, then Flexeril 10 mg TID PRN), and Tylenol. At the initiation of HBO2 treatments, the patient described at least daily pain episodes lasting approximately 1 h that began with a burning sensation in the left anterior chest, progressing to cramp-like pain involving his entire left side. The pain was consistently triggered by airplane flights, with no other obvious aggravating or relieving factors. It variably kept the patient awake at night. HBO2 treatments were administered at 2 atmospheres absolute (ATA) for 90 min each, the protocol routinely used at our multiplace hyperbaric chamber facility for non-emergent diagnoses and used by our group in prior research protocols for the treatment of traumatic brain injury. The initial plan was for 40 treatments based on the report in post-stroke patients to be effective for inducing late neuroplasticity (11) and that showed improvements in NIHSS, activities of daily living (ADL), and quality of life (QoL). However, due to family circumstances and personal obligations, the patient had to take three breaks in the therapy (41, 90, and 28 days), resulting in a treatment course of 100 HBO2 sessions over 11+ months, extended primarily due to ongoing progressive improvement.

The patient continued gabapentin 300–600 mg TID throughout the course of HBO2, required less Tylenol (initially multiple times daily to several times/week), replaced baclofen with Flexeril, and eventually stopped both.

After the initial 10 HBO2 treatments, the patient noted significantly reduced pain during the acute episodes, rated at 4–6/10 compared to his baseline of 10/10. The patient also noted the reduced duration of the acute episodes, lasting hours versus days, and decreased intensity of the left arm and leg stiffness that followed each pain episode. The frequency decreased to only two episodes in the first 3 weeks of HBO2 therapy. After 33 treatments, there was a 41-day gap in therapy due to a planned trip. On resuming HBO2, there was a slight loss of prior improvements, and at treatment #34, the patient had one of his worst pain flare episodes with severe left hemi-body pain and usual post-pain stiffness. The patient continued taking gabapentin 300 mg TID (earlier was 600 mg TID), rare use of Tylenol (earlier was 325 mg TID), duloxetine 30–60 mg/day, and Flexeril.

After 37 treatments, the patient was no longer having acute exacerbations and was only experiencing baseline hemi-body stiffness with no pain. The patient continued with gabapentin and duloxetine with the rare use of Tylenol.

After 56 treatments, there was a 90-day break in HBO2 therapy for another trip. On return, there was again a slight loss of prior improvements, and the decision was made to continue

with a total of 80 treatments, at which time the patient had planned a trip lasting 28 days. Notably, the patient consistently had minor flares in symptoms for 2–3 days after airline flights. After the 28-day break, the patient felt that he was continuing to improve, and was given a final of 20 HBO2 sessions, for a total of 100 sessions over a period of 11 months. At the end of the 100 treatments, the patient was completely asymptomatic. He continued with gabapentin at 300 mg TID and duloxetine at 30 mg/day.

Considering that the patient was seen multiple times by various providers during the 11 months between the CVA and HBO2 and had stable, severe symptoms (no significant or sustained improvement), he was encouraged to maintain a stable medication regimen to best assess his response to HBO2 therapy.

Discussion

Thalamic pain was first described by Dejerine and Roussy as, "… severe, persistent, paroxysmal, often intolerable, pains on the hemiplegic side, not yielding to any analgesic treatment" (12). Thalamic pain is characterized by constant or intermittent pain in the hemi-body, contralateral to the thalamic lesion, and is a severe and treatment-resistant type of CPSP that occurs after a cerebrovascular lesion results in thalamic stroke.

Treatment remains difficult, and the prognosis is typically poor. Possible pathogenesis includes central imbalance, central disinhibition, central sensitization, other thalamic adaptive changes, and local inflammatory responses (1).

PS-TP may develop immediately after a stroke, as in this case report. Up to 40% occur within a month of the stroke, over 40% have symptoms between 1 and 12 months, and sometimes develop 1 to 6 years post-stroke (2, 13, 14).

Right-sided stroke lesions were more commonly associated with the development of PS-TP than left-sided lesions (15).

In a series of patients with thalamic infarcts, only lesions in the ventral posterior part of the thalamus caused CPSP (16).

CPSP can develop after both hemorrhagic and ischemic lesions of the CNS. A lesion in the CNS results in anatomical, neurochemical, excitotoxic, and inflammatory changes, all of which might trigger an increase in neuronal excitability (17) that might lead to chronic pain.

Treatments for thalamic pain syndrome

Treatment of CPSP is usually a combination of several drugs. Commonly recommended first and second lines of pharmacological therapies include traditional medications, e.g., tricyclic antidepressants, SSRIs, SNRIs, anticonvulsants, and opioid analgesics. Anticonvulsants have several mechanisms of action, including reducing neuronal hyperexcitability (3).

Non-pharmacological interventions, such as transcranial magnetic or direct current brain stimulations, vestibular caloric stimulation, epidural motor cortex stimulation (MCS), and deep brain stimulation (DBS), were effective in some case studies and can be recommended in the management of therapy-resistant PS-TP (1). Intracranial neurostimulation for pain relief is most frequently delivered by stimulating the motor cortex, the sensory thalamus, or the periaqueductal and periventricular gray matter. The stimulation of these sites through MCS and DBS has proven effective for treating a number of neuropathic and nociceptive pain states that are not responsive or amenable to other therapies or types of neurostimulation (18).

Recent data suggest that the therapy may have value for treating post-stroke pain, central pain syndromes, and peripheral deafferentation pain (19, 20). An "insertion effect" has been reported, in which the placement of DBS leads provides pain relief even when the leads are not activated (21).

Cilostazol, a selective and potent inhibitor of phosphodiesterase (PDE) 3A, likely increases the level of cAMP and has been reported to be effective (5).

Mexiletine, an orally active antiarrhythmic agent, produced improvement in eight of nine thalamic pain syndrome patients over 4 weeks (22).

In a case report, multidrug-resistant thalamic pain was successfully alleviated by modulating the contralateral thalamic pathway with ipsilateral stellate ganglion block (7).

A patient who developed CPSP 5 years after a right lenticular-capsular thalamic stroke was treated with 2 weeks of mirror therapy with a reduction in pain that was maintained at the 1-year follow-up (6).

Two cases of thalamic syndrome were treated with weekly, increasing doses of naloxone infusions successfully relieved pain for up to 6 months (4).

There is a growing body of evidence showing benefits with HBO2 in post-stroke patients, including post-stroke memory impairment (23), cognitive impairment (24), traumatic brain injury (25, 26), and chronic neuropathic pain (27).

A prospective, randomized, controlled trial of 74 patients, all of whom suffered a stroke 6–36 months prior to treatment with HBO2. The treatment protocol was 40 sessions (5 days/week) at 2 ATA. The authors concluded that HBO2 can lead to significant neurological improvements in stroke patients, even in chronic late stages. Improvements were measured by neurologic evaluation with NIHSS, brain functional imaging (SPECT), and QoL evaluations. The improvements support the idea that neuroplasticity can be activated months to years after stroke with proper stimulation, such as HBO2. This and other studies reveal that many aspects of the brain remain plastic even in adulthood (11). HBO2 can initiate cellular and vascular repair mechanisms and improve cerebral vascular flow. At the cellular level, HBO2 can improve neuronal and glial cell mitochondrial function, improve blood–brain barrier and inflammatory reactions, reduce apoptosis, alleviate oxidative stress, increase levels of neurotrophins, and nitric oxide, and upregulate axon guidance agents. HBO2 may promote the neurogenesis of endogenous neural stem cells (28).

In a review of four studies, the authors concluded that HBO2 is effective for ischemic strokes (26).

HBO2 can exert neuroprotective effects through multiple pathways in acute ischemic stroke (AIS), with consequent salvage of neurological function.

The three phases of potential HBO2 benefit in ischemic stroke are pretreatment, early (limited to 12 h from the time of onset of AIS), and recovery from neurological damage in the chronic phase (29).

In a large retrospective 2020 study including chronic stage post-stroke patients at least 3 months post-injury (median 1.5 ± 3.3-year post-stroke), HBO2 was found to induce significant improvements in all cognitive function domains. In 50 patients (30.86%), the stroke was subcortical. There were no significant differences in HBO2 effects on subcortical strokes compared to cortical strokes (30).

In a clinical study, six patients 1–2 years post-stroke with a stable baseline were treated with HBO2 at 2 ATA X for 60 min for a total of 40 treatments over a 12-week period. The authors reported improvements in cognition, gait velocity, upper extremity mobility, sleep, and overall recovery that were maintained for up to 3 months (31).

A review of HBO2 in acute stroke discussed physiological effects that include ameliorating AIS-induced brain tissue hypoxia, stabilizing the blood–brain barrier, decreasing intracranial pressure, and relieving cerebral edema (32).

HBO2 alleviates neuroinflammation through effects that include reduced inflammatory enzymes and inhibition of neutrophil infiltration and matrix metalloproteinases (MMP-9).

HBO2 can inhibit apoptosis and have neuroprotective effects. Mechanisms include increased expression of neurotrophic and nerve growth factors, mobilization and migration of pluripotent mesenchymal stem cells, proliferation of astrocytes, inhibition of the secretion of microglial cells, and improving outcomes for neurological and motor functions.

The timing of HBO2 in AIS may be an important factor in efficacy. Studies suggest that the ideally, HBO2 should be initiated within the first 3-5 hours post ischemic stroke (33, 34) and has been shown to be beneficial in the chronic phase (11).

HBO2 was shown in a retrospective study of 162 patients to induce significant improvements in all cognitive domains, even in the post-stroke late chronic stage. There were no significant differences in the HBO2 effect on cortical strokes compared to subcortical strokes. Participants were treated at 2 ATA for 90 min (with 5-min air breaks every 20 min) for 40–60 sessions. The median time from stroke to HBO2 was 1.5 ± 3.3 years. Neuroplasticity is induced by two main physiological effects: increasing tissue oxygenation and repeated oxygen level fluctuations, which increase HIF-1a. This, in turn, triggers regenerative processes in the metabolically injured brain areas (30).

HBO2 can improve cognitive functions in patients with mild cognitive impairment. Elevated humanin levels (mitochondrion-derived neuroprotective peptide) were reported in vascular dementia patients treated with HBO2. Maintenance of HBO2 will likely be needed for progressive neurodegenerative diseases (24).

Mechanisms of the neuroprotective effects of HBO2 in stroke patients include stabilizing the blood–brain barrier, improving metabolism, reducing brain edema, alleviating post-stroke neuroinflammation, inhibiting post-stroke apoptosis, improving anoxic area microcirculation, and suppressing ischemia–reperfusion injury (35).

The fact that the patient consistently experienced exacerbations of symptoms during and for 1–2 days after each airline flight strongly suggests that the neurological injury is sensitive to hypoxia, hypobaric pressure, or both. This same observation has been made in other clinical scenarios and was the basis for an experiment on the effect of commercial airline cabin pressure/hypoxic exposures incurred on evacuation flights of injured U.S. military members from Iraq. That experiment in acute brain-injured animals demonstrated amplification of the acute brain injury by hypobaric exposure to 8,000 feet altitude despite the correction of hypoxia (36). The patient's sensitivity to hypobaric exposure at cabin altitude suggests that the opposite, exposure to increased pressure achieved with HBO2 therapy, is consistent with the observed clinical improvements.

Study limitations

Baseline assessments were retrospective, based on the patient and his spouse's recollection of symptoms during the year prior to initiation of HBO2 therapy.

It is unknown whether the patient would have responded differently without the interruptions in the HBO2 therapy.

A definite limitation in the case report is the short (1 month) follow-up. Fortuitously, the symptomatic responses noted during the multiple patient-imposed breaks in therapy, might offer some insight, and imply that the patient could require periodic HBO2 to maintain benefit, as has been suggested in the HBO2 treatment of other brain pathologies.

Questionnaires were completed for only 80 out of the 100 treatments. However, the patient was entirely asymptomatic at the end of HBO2 therapy and had no adverse effects or complications from HBO2 treatments.

Study strengths

Multiple other pharmacologic and non-pharmacologic therapies have reported benefits in thalamic pain syndrome, but none have been attempted in this patient. There were no significant changes in the pharmacologic treatments during the study except for a reduction in pain medications. This allowed for clinical improvements to be attributed to the HBO2 intervention.

Reports of HBO2 mechanisms of action and efficacy in stroke and other brain pathologies support the clinical benefit realized in this case report and are consistent with a regenerative improvement in the chronic stages (1-year post-CVA in this case). HBO2 therapy is a potential treatment option for thalamic pain syndrome. The specific mechanisms of HBO2 therapy need to be further studied and explored.

Patient perspective

I am glad that my wife suggested trying the HBO2 therapy. My symptoms started at the time of the cerebral angiogram (December 2021) but became most intense approximately 9 months later (September 2022), with only partial relief from medications. With each pain episode, I worried whether it would get worse. The year of HBO2 really relaxed my body, and it is the best I have felt since September 2022. The symptoms improved with each new course of HBO2. Overall, the entire year of HBO2 treatments was the best I have felt since onset; the episodes were

less painful, shorter, and did not keep me from doing what I wanted to do.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JS: Writing – original draft, Writing – review & editing. NK: Writing – original draft. PL: Writing – original draft, Writing – review & editing. RG: Writing – review & editing.

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Hyperbaric oxygen therapy for the treatment of hypoxic/ischemic injury upon perinatal asphyxia—are we there yet?

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Birth asphyxia and its main sequel, hypoxic-ischemic encephalopathy, are one of the leading causes of children's deaths worldwide and can potentially worsen the quality of life in subsequent years. Despite extensive research efforts, efficient therapy against the consequences of hypoxia-ischemia occurring in the perinatal period of life is still lacking. The use of hyperbaric oxygen, improving such vital consequences of birth asphyxia as lowered partial oxygen pressure in tissue, apoptosis of neuronal cells, and impaired angiogenesis, is a promising approach. This review focused on the selected aspects of mainly experimental hyperbaric oxygen therapy. The therapeutic window for the treatment of perinatal asphyxia is very narrow, but administering hyperbaric oxygen within those days improves outcomes. Several miRNAs (e.g., mir-107) mediate the therapeutic effect of hyperbaric oxygen by modulating the Wnt pathway, inhibiting apoptosis, increasing angiogenesis, or inducing neural stem cells. Combining hyperbaric oxygen therapy with drugs, such as memantine or ephedrine, produced promising results. A separate aspect is the use of preconditioning with hyperbaric oxygen. Overall, preliminary clinical trials with hyperbaric oxygen therapy used in perinatal asphyxia give auspicious results.

KEYWORDS

apoptosis, brain, hyperbaric oxygen therapy, microRNA, oxidative stress, perinatal hypoxia-ischemia

Introduction

Perinatal asphyxia is a pathological condition caused by impaired blood flow to organs (due to hypoxia-ischemia), before, during, or after birth. Hypoxic–ischemic encephalopathy (HIE), occurring upon hypoxia-ischemia (HI), is a major contributor to global child mortality and morbidity. The prevalence of birth asphyxia is approximately 2–6 per 1,000 live-born infants. As many as 15–25% of them die, while 25% are diagnosed with permanent neurological damage (1).

The mechanism underlying perinatal brain injury after HI originates as an interruption of placental blood flow followed by impaired gas exchange, causing deficits in oxygen and metabolic substrate delivery to the child's central nervous system. These irregularities lead to energy failure characterized by decreased ATP production and an increase in lactate concentration, resulting in systemic acidosis. Energy deficit initiates a cascade of intracellular

events leading to neuronal cell death. The first stage is the depolarization of neurons and glia and the release of excitatory amino acids, mainly glutamate. Activation of NMDA receptors, the critical group of ionotropic glutamate receptors, triggers an excitotoxic cascade (excessive exposure to glutamate) that generates reactive oxygen (ROS) and subsequent oxidative cell damage and initiates inflammation and apoptotic processes. The composition and activity of immature brain NMDA receptors differ from those identified in the adult brain, and their sensitivity to HI conditions increases excitotoxic damage (2). These intracellular mechanisms can continue their destructive activity resulting in myelin deficits, reduced plasticity, and delayed neuronal death. This process can continue for days or even years after the initial HI insult.

Therapeutic hypothermia initiated within a short time after birth was, for a long time, the only clinically available intervention for moderate and severe cases of HI. The treatment is often supported by medication to regulate blood pressure and control seizures, and dialysis to support the kidneys. However, these modalities are not efficacious enough to significantly improve the survival outcome and prevent infant brain damage. Moreover, it was reported that 64%, of children with mild HIE treated with hypothermia still developed moderate HIE (3). Therefore, many experimental therapies are still under investigation. Hypothermia is often combined with inhibitors of calcium channels and ionotropic glutamate receptors (topiramate, memantine, magnesium sulfate, 50% xenon), erythropoietin, or phenobarbital (4, 5). Hyperbaric oxygen therapy (HBOT) is one of the propositions for HI treatment (6).

The efficacy of HBOT proposed in the 1960s as an experimental treatment of neonatal HI, was controversial due to the inconsistency of outcome benefits in early trials (7–9). Since then, however, HBOT has demonstrated effectiveness in perinatal asphyxia at <3.0 ATA not only due to the neuroprotective effect but also the apparent lack of side effects such as retinopathy (10–12). The neuroprotective mechanism of HBOT is related to an increase in the oxygen reservoir at the cellular level and triggering/supporting intracellular defense mechanisms (Figure 1). However, some HBOT-mediated neuroprotective effects in neonates are controversial, as results from different groups are inconsistent. This article will thus focus on intracellular HBO-activated defense mechanisms. The bulk of experimental data comes from the most universally accepted model of neonatal hypoxia-ischemia according to the Rice-Vannucci procedure in 7-day-old rats (13). Furthermore, we will discuss data from clinical trials where HBO therapy was used to treat neonatal patients and the current clinical use of HBOT in hypoxic–ischemic neonates.

The effects of HBO treatment in experimental hypoxia-ischemia

Therapeutic window

The established optimal therapeutic window for HBO intervention in neonatal hypoxia-ischemia is 6 h from the HI occurrence, and experimental HI in neonatal rats fits into this time frame. Within this therapeutic window, HBO was highly effective in reducing brain damage, predominantly neuronal loss in HI-sensitive regions, such as the CA1 area of the hippocampus and cerebral cortex (14–16).

Wang et al. showed that HBO treatment, administered for 60 min and repeated for 7 consecutive days, could be delayed by 12 h from the time of HI (14). HBO therapy started 48 h after HI did not instigate brain protection (17), although it was also shown that 60 min of HBO treatment started 24 h and repeated for 14 days might improve brain functional outcome and reduce neuronal death in neonatal rats in the model of intrauterine HI (18). Unfortunately, reports systematically determining the maximum duration of HBOT use are lacking. However, depending on the HBO treatment protocol, regarding the starting point of the treatment, the duration of a single session, and the number of treatments, results suggest that the time window can be expanded beyond the assumed 6 h.

Taking into account the limited time for effective intervention after perinatal asphyxia and anticipating complications that may result in HI, scientists look for alternative methods by which brain damage can be prevented, in addition to the already existing effective therapy of HI. These considerations prompted the concept of preconditioning: a treatment that uses noxious stimulus short of triggering the damage but strong enough to instigate defense mechanisms in the tissue poised to be exposed to damaging factors. Murry and colleagues first introduced the concept, documenting a protective effect of brief ischemic episodes applied before subsequent sustained heart ischemia in dogs (19). Since then, ischemic preconditioning has been thoroughly studied in different cases of heart and brain ischemias (20). HBO preconditioning (HBO-PC) also became the subject of research and its beneficial effects in neuroprotection were reported (21, 22). However, only three studies have validated the efficacy of HBO-PC in preventing brain injury after HI. A single session of HBO-PC, conducted at 2.5 ATA for 2.5 h (23, 24) as well as three days of sessions at 2.5 ATA for 2.5 h each (25) significantly reduced brain damage, oxidative stress, and apoptosis after neonatal HI in a rat model. Despite the authors' claims of the safety and efficacy of HBO-PC for neonatal HI, its introduction into clinical practice is problematic as the occurrence of perinatal hypoxia is usually unpredictable, and in such cases, obstetricians usually deal with them more conventionally.

Oxidative stress inhibition

One of the major issues related to perinatal HI brain injury is oxidative stress resulting from impaired mitochondrial functioning that leads to the overproduction of reactive oxygen species (ROS), such as singlet oxygen, hydroxyl radical, superoxide anion, hydrogen peroxide, and nitric oxide. Excitotoxicity caused by excessive release of excitatory neurotransmitters such as glutamate sets the process in motion. Mechanistically, in response to excitatory postsynaptic currents, mitochondria uptake excess cytosolic calcium, producing high ROS concentrations during disturbed mitochondrial respiration (26). Neuronal nitric oxide synthase (nNOS) can have a deleterious effect after HI, leading to the additional accumulation additional amount of reactive nitrogen species (RNS), e.g., peroxynitrite (27, 28). Three main antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) supported by glutathione, and catalase are mobilized to counteract the toxic effects and maintain the delicate balance of ROS in neurons (29).

An interesting review by Schottlender et al. summarized the effects of HBO treatment on mitochondrial function and oxidative stress, comparing protocols with varying oxygen pressure and time,

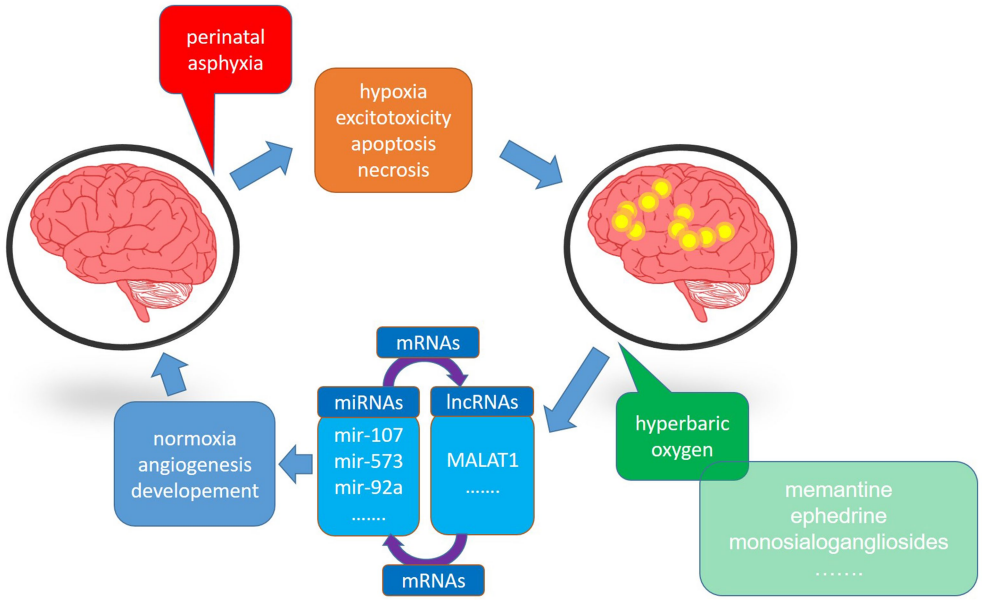

FIGURE 1
The schematic diagram showing the impact of hyperbaric oxygen therapy on the deleterious effect of hypoxia-ischemia (HI) on the brain. HI induces energy deficiency, apoptosis, necrosis, and loss of brain functions. On the other hand, hyperbaric oxygen, supplied in an appropriate therapeutic window, restores normal cellular functioning, significantly reducing brain injury. Both processes exert their activities through miRNAs and lncRNAs.

and exposure frequency in different pathologies and healthy objects (30). In conclusion, a short HBO treatment (1–5 consecutive sessions) reduced mitochondrial function, while long expositions (20–60 consecutive sessions) improved mitochondrial parameters significantly. However, reports on the effect of HBOT used in experimental HI are not included and generally are sparse.

Few available publications brought valuable information on the effect of HBO therapy in experimental HI on the development of oxidative stress in neonatal rat brains. Several sessions of HBOT started up to 6 h after HI significantly reduced ROS levels in the brain (31). Increased SOD concentration measured in hippocampal homogenates derived from neonatal hypoxic–ischemic rats subjected to HBOT compared to the HI group was reported by Chen et al. (32). Moreover, the concentration of malondialdehyde (MDA), one of the critical end-products of lipid peroxidation, was significantly lower than in untreated animals.

Interestingly, Zhao et al. report increased glutamate accumulation and glutamate-induced oxidative stress in healthy rat puppies exposed to hyperoxia, accompanied by a decreased glutamate transporters expression (33). However, HBOT performed on ischemic adult rats significantly reduced glutamate release and ROS formation (34). Moreover, experimental data showed that SOD expression and anti-apoptotic Bcl-2 significantly increased in the brains of healthy gerbils subjected to five sessions of HBOT at 2 ATA (35), indicating different effects of HBO treatment depending on experimental conditions. Moreover, these data also suggested that the immature brain in HI conditions may respond to the HBOT differently than the one not exposed.

Barely two publications reported the effect on oxidative stress defense factors measured in the blood of neonates subjected to HBOT. Zhou et al. assessed the effect of HBO applied at different pressures (1.4–1.6 ATA, repeated for seven days) on changes in peroxidation and antioxidant levels in the serum of 60 neonates with HIE (36). SOD level was significantly higher and the serum concentration of MDA was significantly lower compared to the control group. Excessive activity of nitric oxide synthase (NOS) and nitric oxide (NO), apparent after HI and causing similar damage to ROS, was also significantly lower in examined serum samples of neonates.

Some authors (37) presenting the results of blood tests of 14 asphyxiated full-term newborns treated with HBO, reported no significant changes in SOD and catalase content; however, they noted a significant increase in the activity of GPx and reduction in ROS content.

The information cited above indicates that HBOT used in HI significantly stimulates antioxidant defense, which makes it an important element in stopping the development of oxidative stress and brain damage.

Apoptosis inhibition

Apoptosis is a critical pathway of neuronal cell death after HI and many studies have aimed to prevent or reduce the development of apoptotic processes after HI.

The HBOT significantly reduced the percentage of apoptotic cells in most infant rat brain regions sensitive to HI, such as the hippocampus and cortex (31, 38, 39). Moreover, the HBO treatment 60 min after HI significantly reduced the level and activity of the pro-apoptotic caspase-3 (38, 39). Calvert et al. reported a significant amount of cleaved poly (ADP-ribose) polymerase (PARP) a caspase-3 substrate, which is considered to be a hallmark of apoptosis, in the hippocampus and cortex upon HI; however, HBO treatment significantly decreased cleaved PARP content in both regions (38).

Mitochondria release apoptosis-inducing factor (AIF), an essential element of caspase-independent apoptosis, in response to HI; however, the HBO treatment significantly attenuated such mitochondrial release and inhibited the caspase-independent neuronal cell death (39). It was also shown that HBO-PC significantly reduced the activity of caspase-3 and caspase-9 in the cortex and hippocampus compared to untreated animals in the neonatal HI rat model (23).

Hyperoxia elicits injury to premature lungs and retina and there is an opinion that it may cause brain damage if used in newborns; however, data presented above suggest that HBO treatment may effectively decrease HI-induced apoptosis, thus preventing brain damage. This effect of compressed oxygen may be beneficial only in hypoxic–ischemic conditions.

Neural stem cell activation

The discovery of neural stem cells in the mammalian brain suggested regenerative potential for areas affected by HI injury, opening up new therapeutic possibilities. The HI significantly increased the number of neural stem/progenitor cells in the subventricular zone of 7-day-old rat puppies subjected to experimental birth asphyxia (40). As HI occurs during intensive brain growth and neurogenesis, even increased by HI conditions neural cell proliferation is insufficient to repair any damage and restore development completely. Thus, amplifying such a response could be a valid therapeutic approach. Of note, HBO enhanced the proliferation of neural stem cells in the subventricular zone of neonatal rats suffering from hypoxic–ischemic brain damage (41). Moreover, HBOT applied to HI neonatal rats significantly increased the levels of Wnt-3, the protein whose signaling pathway is associated with neurogenesis in neural stem cells. Similarly arose levels of nestin, the protein expressed in dividing cells during the early stages of the central nervous system development (42). These findings were thus in accord with previous reports on HBOT-mediated significant reduction of the ischemia-induced downregulation of neurotrophin-3, which promotes the survival of progenitors and their differentiation into neurons in the forebrain of rats (43).

Interactions with miRNAs

MicroRNAs (miRNAs) are small, ~22 nucleotide RNA molecules mainly responsible for the negative regulation of transcription of mRNAs either via their destabilization or translation blockade. Neurons are particularly enriched in microRNAs, with as much as half of them expressed in this cell type (44, 45). Recently, miRNAs have gained attention as potential diagnostic markers and therapy targets (46, 47). Research on perinatal HI and HIE identified a multitude of miRNAs involved in these processes, mainly regarding humans (umbilical cord blood—UCB and peripheral blood), mice, and piglets (UCB, carotid artery blood, and brain). Although direct reports on the involvement of miRNAs in HBO applied therapeutically upon birth asphyxia are lacking, we discuss this topic here. Several of the miRNAs identified as being important in neonatal HI, brain ischemia (middle carotid artery occlusion model) (48, 49), and HBO (including model of hyperoxia in retinopathy) are common for the all three (Figure 2).

Prominent among forms of brain injury after HI is cerebral white matter damage (WMD). HI-induced dysregulation of Wingless and Int-1(Wnt)/β-catenin signalling disrupts and delays normal myelination (50). HBOT administered within 12 h after HI alleviated WMD (51); while activating Wnt signaling (42). Many miRNAs regulate the Wnt pathways (canonical and non-canonical) at different levels (52–54). Both, mir-374a and mir-376c, altered in neonatal UCB of neonates upon suffering from HI and HIE, can regulate the Wnt/β-catenin pathway (55, 56). The non-canonical Wnt pathway is influenced by mir-128, mir-148a, mir-151a, mir-181a, and mir-181b, all upregulated in the blood of piglets subjected to HI (57, 58). Interestingly, mir-1264, upregulated in the mouse brain immediately after HI, can lead to apoptosis through Wnt/β-catenin signaling (59). The involvement of these miRNAs in the HBOT effect on Wnt signaling is highly probable.

The exposure of nucleus pulposus cells to HBOT (100% oxygen at 2.5 ATA) upregulated mir-107. This microRNA not only inhibits its direct target Wnt3a (60) but also suppresses the HMGB1/RAGE/NF-κB pathway, thus deactivating metalloproteases (MMP-3 and MMP-9), calcium-dependent zinc-containing endopeptidases that contribute to the disruption of the blood–brain barrier and extracellular matrix (61). Mir-107 was downregulated in UCB of human patients suffering from HI (56), as well as downregulated in blood and upregulated in the brains of rats subjected to transient focal cerebral ischemia (48); however, other studies found the opposite direction of changes (62, 63).

Another candidate microRNA to regulate the Wnt/β-catenin pathway, mir-185, is downregulated in UCB upon neonatal asphyxia (56), in the blood of rats subjected to brain ischemia (48), and in the blood of young stroke patients (62); however, mir-185 was upregulated when measured in whole brains (48). Notably, mir-185 inhibits glutathione peroxidase (GPx), an enzyme crucial in the antioxidant cell defense (64). Mir-107 also can regulate MAPK and subsequently NF-κB, thus influencing the inflammatory processes in the cells (65).

Many miRNAs can influence the activity of inflammation pathways; NF-κB impacts the expression of many miRNAs (66). MiRNAs altered upon HI that are central for the regulation of the Wnt/β-catenin pathway can also affect the activity of pro-inflammatory MAPK/NF-κB, including mir-331-5p downregulated in UCB after HI, mir-2137, and mir-137 upregulated after 24 h in mouse brain exposed to HI (59, 67, 68).

Li and co-workers reported that proteins expressed differentially in rat spinal cord injury upon HBOT were linked to mir-9a-5p and mir-125b-5p (69). Mir-9a-5p induced recovery after spinal cord ischemia–reperfusion injury through Notch signaling which is critical for developing and maintaining tissue homeostasis (70), while mir-125b inhibited SOD (64). Interestingly, mir-125b-5p was downregulated 48 h after birth in the venous blood in moderate/severe HIE with poor outcome (68) but upregulated in the brains of rats subjected to cerebral ischemia (48).

MiRNAs are also involved in the process of apoptosis after HI, including those engaging the Wnt pathway and inflammation processes (71). Those include mir-7, mir-128a, or mir-155 (72, 73). Some miRNAs involved in apoptosis were differentially expressed in UCB when comparing the severity of HIE, e.g., mir-98-5p was downregulated and mir-145-5p significantly upregulated in moderate/severe HIE with poor outcome as compared to no/mild HIE (59). Importantly, miRNAs influencing the activity of the Wnt pathway, e.g.,

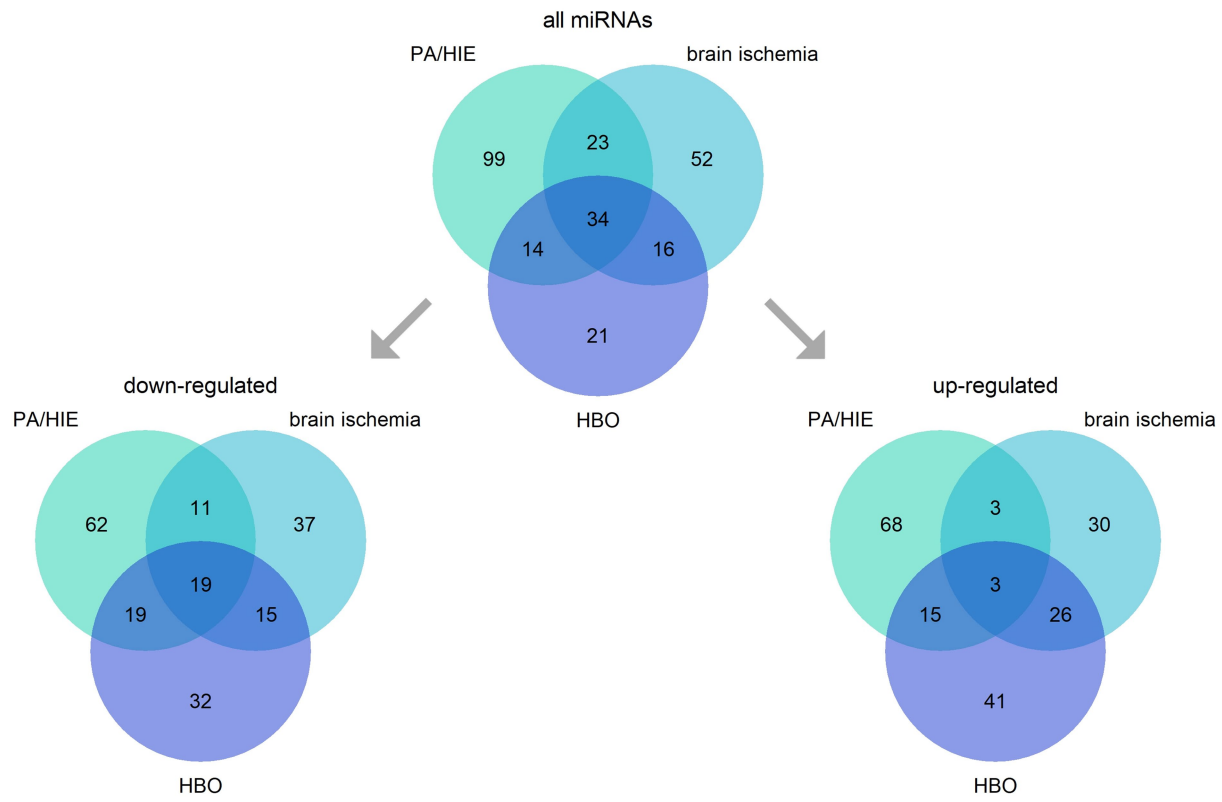

FIGURE 2
Intersections of miRNAs engaged in neonatal hypoxia-ischemia and its consequence —hypoxic–ischemic encephalopathy (PA/HIE), brain ischemia, and hyperbaric oxygen (HBO). The figure presents all miRNAs identified, and division into groups depending on their up-or down-regulation in analyzed conditions.

mir-128, mir-148, mir-151a, mir-181a, mir-181b, mir-374a, or mir-376c-3p, can also regulate apoptosis (55, 58, 67). Mir-573, upregulated after HBOT, was responsible for the repression of Bax mRNA, consequently cleaved forms of caspase-3 and caspase-9 were also decreased (74).

The induction of the long non-coding RNA MALAT1 by HBO in human coronary artery endothelial cells and a rat model of acute myocardial infarction downregulated anti-angiogenic mir-15a and mir-92a (75, 76). Surprisingly, the mir-15a was downregulated in UCB of infants suffering from HI (56) but upregulated, along with mir-92a, in young stroke patients (62). The mir-155 was upregulated in the brains of mice 72 h after hypoxic–ischemic brain injury (67) and in rats 24 h after traumatic brain injury (63). Mir-155, which influences angiogenesis by repressing cysteine-rich angiogenic inducer 61 (CYR81, CCN1), produced in vascular cells and involved in redox homeostasis by inhibiting NOS and transcription factor ELK3, was upregulated in the brains of mice 72 h after HI brain injury (64, 67, 77, 78).

MiRNAs targeting hypoxia-inducible factor 1-alpha (HIF1α), specifically mir-137 and mir-335, were found to be upregulated 24 h and then downregulated 72 h after HI in mouse brains (67). Interestingly, mir-137 can inhibit NADPH oxidase (NOX), while mir-335 regulates NOS and SOD expression (64), thus being involved in redox homeostasis in cells. Other miRNAs essential for downregulating hypoxic response and downregulated in UCB, include mir-210, which has the potential to discern the severity of HIE, and mir-181b, which was significantly lowered in moderate and severe HIE (79). Thus, the involvement of these miRNAs in the HBOT effects is likely, but further research will be required.

Combining HBOT with other therapies

Most studies conducted on experimental HI, as well as in reported clinical cases of neonatal HI, in which HBO therapy was applied, showed the beneficial effects of its use. However, researchers aiming to increase the effect combine HBO therapy with neuroprotective drugs used in clinical practice.

Memantine is a non-competitive, low-affinity blocker of NMDA receptors, and inhibiting these receptors has been considered a valid therapeutic approach for many years. In clinics, memantine is used to treat Alzheimer's and Parkinson's diseases; therefore, its combination with HBO in HI therapy seemed to be promising. The results presented by Gamdzyk and coworkers failed to demonstrate an additive increase in neuroprotection in experimental HI on 7-day-old rats upon combining HBOT with memantine 1 h or 6 h after HI insult (31). On the contrary, combining the treatments resulted in lower neuroprotection than the effects of HBO or memantine applied alone. However, Wang and coworkers reported that a combination of HBO and memantine treatments not only significantly reduced brain damage, oxidative stress, and inflammation in the brains of adult rats subjected to transient focal cerebral ischemia but also prolonged the

therapeutic window from 6 to 12 h after reperfusion (80). These conflicting results may arise from differences between the expression of NMDA receptor subunits in developing and adult brains (31, 81, 82).

Ephedrine is a well-known sympathomimetic agent and a traditional Chinese herbal medicine and has been used to treat asthma, obesity, and narcolepsy. It has also been used to treat or prevent hypotension. The mechanism of ephedrine action relies on increasing the stimulation of the postsynaptic α and β adrenergic receptors by norepinephrine (83). Ephedrine crosses the blood–brain barrier and stimulates the nervous system, and some amphetamine-and methamphetamine-like responses were attributed to ephedrine. However, its medical use in small doses is accepted to treat several medical conditions. Ephedrine was also proposed as a stimulant to enhance the endurance and stamina of military service members (84). Chen and coworkers reported the neuroprotective effect of ephedrine alone and with HBO in newborn rats subjected to HI (85). In the experimental group treated with an ephedrine/HBOT combination, decreased brain edema and neuronal loss, inhibition of apoptosis, and improvement in memory formation were apparent when compared to controls and single-agent treatment. The results indicated that the combination had a synergistic effect; however, this combined therapy's precise molecular and cellular mechanisms require further investigation.

Earlier publications indicated that monosialogangliosides significantly reduced white matter damage in experimental ischemia conducted in rats (86). It was also reported that in the clinical treatment of newborns suffering from HIE monosialogangliosides reduced serum apoptotic factors and increased serum content of SOD, GPx, brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) while maintaining the stability of the internal environment (87). The use of monosialogangliosides as adjuvant treatment had also beneficial effects in the improvement of neurological outcomes (88). Interestingly, a meta-analysis performed by Gong and coworkers on using HBOT in clinical practice as adjuvant therapy for neonatal HIE mentioned 26 successful cases of combining HBOT with gangliosides (89).

Interestingly, the combination of HBO with erythropoietin in experimental rat spinal cord injury (90) and in humans diagnosed with cardiac insufficiency (91) offered promising results on combining HBO treatment with other agents.

The clinical application of HBO therapy

HBOT is currently recommended for treating 14 conditions, including carbon monoxide poisoning, air embolism, diabetic foot, severe anemia, and decompression sickness (92). These treatments are well-accepted in adult patients and approved by the Undersea and Hyperbaric Medical Society (93). However, therapy for neonatal and pediatric patients is still treated with great reserve.

The first report on the use of HBO in the treatment of neonatal HI appeared in 1963. Hutchison and coworkers described that 35/65 (54%) children survived thanks to HBO therapy (8). However, this report was significantly criticized, and the authors were accused of using risky, not thoroughly proven therapy with insufficient preliminary animal research (7, 94).

Since then, the research into the use of HBOT in brain ischemia has progressed and a large volume of data has been published indicating its neuroprotective effects, including reports on the neuroprotective effects of HBOT in animal models of birth asphyxia. Thus, reports on the beneficial effects of HBO in brain ischemia, performed on animal models and clinical trials, have encouraged medical professionals in some countries to apply it in clinical practice. Scientists from the former USSR in the early 1980s reported almost 2,000 cases of the effective use of HBOT in the treatment of newborns suffering from birth asphyxia (Proceedings of VIIth International Congress of Hyperbaric Medicine, 1981). Articles published in Russian by Kostin, Baiborodov, and co-workers (37, 95–97) indicated the continuous use of HBOT in the clinic. Some reports from Mexico described the successful use of HBOT in treating term neonates with moderated HIE (98) and neonates with HIE and necrotizing enterocolitis (99). Despite the visible clinical improvement and no signs of hyperbaric oxygen side effects, the authors indicated technical problems due to the lack of intravenous pumps capable of working in hyperbaric conditions.

Nevertheless, technical progress has allowed the construction of large hyperbaric chambers that can accommodate a patient and qualified medical personnel. There are also specialized intravenous pumps and respirators adapted to work in hyperbaric conditions for newborns available on the market. Patients can also be monitored with ECG and with transcutaneous oxygen monitor (100). Moreover, for the additional safety of patients, including neonates, some countries have introduced specialized courses for nurses working with hyperbaric chambers. For example, the first hyperbaric nurses in the United States were certified in the late 1980s (101).

Apparently, HBOT is currently used mainly in China. Liu and coworkers published an extensive review article in which they discussed 20 trials, mainly from Chinese sources, in which HBOT at various doses, and in several cases with additional treatments, was used to treat hypoxic–ischemic neonates (100). The information presented in this review indicates that, although the use of HBOT in China is still controversial, this treatment reduced mortality and neurological sequelae in term neonates with HIE. Fifteen years later, Gong and coworkers published a systematic meta-analysis analyzing the use of HBOT as adjuvant therapy for neonatal HIE (89). They analyzed 46 selected randomized controlled clinical trials, including 4,199 patients with neonatal HIE. All of the trials were performed in China. Studies accomplished as part of this meta-analysis indicated that patients from the HBOT group exhibited a significantly better response to treatment than the control group. The incidence of sequelae was reduced, and the neonatal behavioral neurological assessment (NBNA) score of the HBOT group was significantly higher than that of untreated children. This meta-analysis also highlighted the lack of systematization of HBOT regarding pressure, exposure time, and duration of therapy. However, using a subgroup analysis, the authors determined the best treatment protocol for HBOT of neonates with HIE: (1) HBOT pressures 1.4–1.6 ATA are safe and effective for neonates suffering from HIE; (2) daily oxygen intake for 30–40 min provides the maximum therapeutic effect for neonates with HIE; and (3) the best therapeutic effect is achieved when the therapy lasts over 30 days. This is the first step towards creating an acceptable procedure for HBOT in the clinic, but given that the clinical trials were conducted in only one country, further larger studies are necessary.

Discussion

Brain ischemia directly links to a plethora of pathologies, including stroke, cardiorespiratory arrest, and birth asphyxia. As it involves a reduced supply of oxygen to the brain, tissue hypoxia triggers vital molecular pathways such as excitotoxicity, oxidative stress, apoptosis, and inflammation that inevitably lead to cell death. Elevating oxygen levels in tissue affected by ischemia thus seems to be a valid approach to preventing neurodegeneration. HBOT is the most effective method of increasing dissolved oxygen concentration in plasma, providing the necessary oxygen supply to ischemic tissue. Breathing oxygen at a pressure of 2.8 ATA increases hemoglobin oxygenation (from 83 to 88%), cerebral blood flow, and regional cerebral oxygenation (102–104). Such conditions can counterbalance the HI-induced changes in the brain. Plentiful evidence obtained from animal studies and clinical trials indicates the beneficial, neuroprotective effects of HBOT used in the treatment of stroke, and traumatic brain injury, and its beneficial effect on ischemia–reperfusion injury (105–108). The authors reported the neuroprotective effect of HBOT resulting in metabolic and molecular rearrangements manifested by improved mitochondrial functioning along with a decrease in oxidative stress (30), acidosis and apoptosis inhibition, anti-inflammatory effects (109), coinciding with improved blood–brain barrier integrity, and reduced platelet aggregation and brain edema (110). Unfortunately, similar reports regarding experimental birth asphyxia, including clinical trials, are scarce. Available evidence indicates that HBOT applied within a short window after the HI reduces brain damage in newborns (16, 17, 31, 38, 89, 100).

Details of molecular mechanisms of neuroprotective HBOT effects come mainly from limited publications on animal models of neonatal HI, focusing on alterations caused by cerebral hypoxia or the antioxidant factors in the newborn blood. These reports show that HBOT significantly reduces HI-mediated ROS production, increases antioxidant enzyme activity, and inhibits apoptosis by restoring the balance between pro-and anti-apoptotic proteins in newborns' brains and blood (Table 1). The activation of neuronal stem cells after HBOT reported in several publications also indicates this therapy's considerable potential in preventing HI-evoked brain injury.

The expression of many miRNAs is affected by HI or HBOT; however, data describing the effect of HBOT applied upon HI on miRNA expression are few. The significant diagnostic and therapeutic potential of miRNAs in many neurological disorders, including perinatal asphyxia and HIE, was strengthened by the recent findings on HBOT. The multitude and variety of the models and protocols used to collect samples diminishes the consistency of results. Multiple miRNAs can influence one biological process, further muddling the picture. The involvement of miRNAs in HBOT-mediated neuroprotection in birth asphyxia deserves further investigation, considering their use as potential biomarkers and therapeutic agents/targets.

In the clinical studies described, in which HBOT was used, clinical improvement in the condition of newborns suffering from HI was assessed using multiple methods, such as ECG and transcutaneous oxygen monitoring during HBO session, outcome indicators such as total efficiency (clinical symptoms, signs, and craniocerebral computed tomography), neuronal sequelae, and the NABA score (89, 98). The accumulated results of these studies indicate that the benefits of HBOT are obvious.

The consensus is that the primary source of beneficial effects of HBOT is an increase in the level of oxygen contained in the blood, especially oxygen dissolved in blood plasma. Indeed, breathing 100% oxygen at a pressure of 2 ATA increases the oxygen diffusion radius up to more than 4-fold. Consequently, the tissue oxygen concentration in the ischemic areas also increases (113).

Increased oxygen partial pressure causes hypothermia, which is desirable in the treatment of HI-affected neonates. Observed hypothermia was only partially the effect of pressure alone (114). However, a reduction of the temperature in the brains of gerbils subjected to ischemia was observed in animals treated with hyperbaric air (HBA), and in animals breathing 100% oxygen (NBO). Only a slight temperature drop was registered, although the oxygen partial

TABLE 1 Compilation of data showing the effect of HBOT used in HI on oxidative stress, apoptosis, and stem cell proliferation.

Affected process	Source	Pressure/time of exposition	Observed effects	References
Antioxidant defense	7-days old rat brains	2.5 ATA, 1,3 or 6 h after HI, 60 min	↓ ROS	Gamdzyk et al. (31)
	7-days old rat brains	2 ATA, 24 h after HI, 60 min/day, 14 days	↑SOD, ↓MDA	Chen et al. (32)
	Human neonates blood serum	1.4–1.6 ATA,?	↑SOD, ↓MDA, ↓NOS, ↓NO	Zhou et al. (36)
	Human neonates blood serum	?	↓ ROS, SOD no changes, ↑ GPx	Baiborodov et al. (97)
Apoptosis	7-days old rat brains	2.5 ATA, 1 h after HI, 90 min.	↓ caspase-3, ↓ AIF, ↓TUNEL positive cells	Liu et al. (39)
	7-days old rat brains	3 ATA, 1 h after HI, 60 min.	↓ caspase-3, ↓ PARP cleavage, ↓ TUNEL positive cells	Calvert et al. (38)
	7-days old rat brains	2.5 ATA, 1, 3 or 6 h after HI, 60 min.	↓ TUNEL positive cells	Gamdzyk et al. (31)
Stem cells proliferation	Neonatal rat brains	2 ATA, 3 h after HI, 60 min/day, 7 days	↑ newly generated neurons	Yang et al. (111)
	7-days old rat brains	2 ATA 3 h after HI, 60 min/day, 7 days	↑ migration of neural stem cells to the cortex	Wang et al. (112)
	7-days old rat brains	2 ATA 3 h after HI, 60 min/day, 7 days	↑ Wnt-3, nestin	Wang et al. (42)

pressure during NBO is two times higher than during HBA (115). Moreover, Fenton and colleagues observed a decrease in body temperature in experiments, where the partial pressure of oxygen in the air at 4 ATA was the same as the partial pressure in the air at 1 ATA (114). These experiments open new questions regarding the mechanism of action of HBOT.

Summary

Considering the multifaceted HBOT effects that can alter multiple HI-instigated molecular processes resulting in neurodegeneration, there is clearly a need for more comprehensive research on the subject. High-quality, randomized clinical trials demonstrating the effectiveness of HBOT in neonates suffering from birth asphyxia will allow including it into clinicians' therapeutic arsenal while shedding new light on the mechanistic intricacies of the approach.

Author contributions

DM: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. JG: Writing – review & editing. ES: Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Case report: Consecutive hyperbaric oxygen therapy for delayed post-hypoxic leukoencephalopathy resulting from CHANTER syndrome caused by opioid intoxication

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Delayed post-hypoxic leukoencephalopathy (DPHL) is a poorly recognized syndrome characterized by neuropsychiatric symptoms following recovery from an acute hypoxic episode. Although most cases are related to carbon monoxide poisoning, some have been linked to excessive opioid use. Opioid intoxication has recently become known for manifesting the characteristic imaging findings involving cerebellar, hippocampal, and basal nuclei transient edema with restricted diffusion (CHANTER) syndrome. Herein, we present a patient with severe disturbances in consciousness who was initially diagnosed with CO poisoning but was later found to have taken excessive tramadol. Magnetic resonance imaging (MRI) in the acute phase revealed abnormal intensities in the bilateral globus pallidus and the cerebellum, indicative of CHANTER syndrome. After intensive care, his level of consciousness was restored. However, around the 3rd week after hospitalization, his consciousness gradually deteriorated and he developed severe neurological symptoms. Another MRI on day 25 revealed a new diffuse white matter abnormality; DPHL was suspected. Cerebrospinal fluid collected on day 28 revealed significantly elevated myelin basic protein levels. Although it was challenging to decide on a treatment plan, hyperbaric oxygen (HBO) therapy trials were initiated on day 58; the patient's condition improved after a series of HBO sessions. MRI revealed gradual shrinkage of the white matter abnormality. A total of 63 consecutive HBO sessions were performed, leading to the successful resolution of the serious neurological symptoms. While the effectiveness of HBO therapy for DPHL remains inconclusive, especially in opioid-related cases, this patient made a remarkable recovery, likely due to the therapeutic effect of improved cerebral blood flow and oxygenation.

KEYWORDS

hyperbaric oxygen therapy, delayed post-hypoxic leukoencephalopathy, delayed neurological sequelae, opioid intoxication, carbon monoxide

1 Introduction

Delayed post-hypoxic leukoencephalopathy (DPHL) is an under-recognized syndrome that manifests neuropsychiatric symptoms after recovery from an acute hypoxic episode (1). The course of the disease may improve or lead to permanent disability. Currently, no effective treatment is available, and supportive care and rehabilitation are the primary focuses. Most cases of DPHL are associated with carbon monoxide (CO) poisoning. However, cases associated with opioid overdoses have also been reported (2).

Opioid abuse disturbs consciousness and brain function. Recently, a clinicoradiological entity related to opioid intoxication involving cerebellar, hippocampal, and basal nuclei transient edema with restricted diffusion has been reported and ascribed the term CHANTER syndrome (3).

Our facility has a multi-person hyperbaric oxygen (HBO) therapy chamber, and we routinely perform HBO therapy on patients, including critically ill patients requiring tracheal intubation. HBO is known for improving various pathological conditions by enhancing the hypoxic environment in the body. HBO is also performed for DPHL after CO poisoning and is sometimes effective. However, its efficacy remains inconclusive. Therefore, HBO is still performed on a case-by-case basis.

Herein, we present the case of a patient who was diagnosed with CHANTER syndrome due to an opioid overdose, followed by DPHL, and treated with consecutive HBO.

2 Case description

A 47-year-old male patient was admitted to our hospital due to severe disturbances in consciousness (Glasgow Coma Scale [GCS] score of 3). Hypoxemia due to impaired consciousness, which required respiratory management with intubation, was observed. Initial magnetic resonance imaging (MRI) revealed abnormal intensities in the bilateral globus pallidus and cerebellum (Figure 1). Although the carboxyhemoglobin level was as low as 1.2 %, CO poisoning was initially suspected based on characteristic MRI findings.

Daily HBO (2.8 ATA, 60 min) therapy was initiated based on the tentative diagnosis of CO poisoning. However, his family later found numerous empty tramadol-acetaminophen packing sheets (tramadol/acetaminophen dose equivalent: 1,575 mg/13,650 mg), which led to the diagnosis of tramadol intoxication. Subsequently, the serum acetaminophen concentration, measured over 15 h post-drug ingestion during transport, was notably high at 10.5 ug/ml, indicating a pharmacokinetic overdose. Tramadol was prescribed by a local physician for lumbago. HBO therapy was performed for 3 days. In addition, acetylcysteine via the gastric tube and intravenous naloxone were administered. His level of consciousness recovered to a GCS score of 14; the patient was extubated.

The patient was hospitalized, wherein rehabilitation was continued. However, his consciousness gradually deteriorated on the 20th day of hospitalization. Another MRI on day 25 revealed a new diffuse white matter lesion, and DPHL was suspected. Cerebrospinal fluid collected on day 32 revealed that the myelin basic protein levels were significantly elevated to 135.5 pg/mL. The patient was bedridden, unable to communicate, and had difficulty in ingesting food. Therefore, gastrostomy was considered.

Weekday HBO (2.0 ATA, 60 min) trials were started on day 58, regarding the routine HBO regimen for DPHL due to CO poisoning. The patient started to speak at the seventh and showed fidgeting at the ninth HBO session; daily activity gradually improved after that. He was able to spend time in a wheelchair and underwent routine HBO with other patients in a multi-person chamber. At the 40th HBO session, his Mini-Mental State Examination (MMSE) score recovered from an unmeasurable level to 15; the patient was able to walk independently. Although HBO was sometimes interrupted, the patient received 63 HBO treatments (Figure 2; Supplementary Video). The patient eventually reintegrated into society. After initiating HBO, MRI revealed a gradual disappearance of white matter abnormality (Figure 3). However, hypoperfusion in the frontal lobe detected on N-isopropyl-p-[^{123}I]-iodoamphetamine single-photon emission computed tomography (^{123}I-IMP-SPECT) that appeared during the course, remained evident (Figure 4).

3 Discussion

We performed consecutive HBO therapy on the patient, leading to the successful resolution of the serious neurological symptoms. However, the effectiveness of HBO therapy in managing opioid-related DPHL remains uncertain.

We present a patient with tramadol intoxication in the initial phase, whose imaging findings of the affected basal nuclei and cerebellum suggested CHANTER syndrome. However, not all the reported features were present. In addition to naloxone administration and systemic management, we performed HBO therapy, which may have exerted therapeutic effects in the acute phase. However, neurological symptoms recurred, and white matter lesions were observed on MRI in the chronic phase. Furthermore, cerebrospinal fluid analysis suggested that myelin was significantly damaged. The patient's condition gradually deteriorated. However, there were no treatment guidelines for opioid-related DPHL, which made deciding on a treatment plan challenging.

Patients with acute CO poisoning present with impaired consciousness; severe cases present with the involvement of the globus pallidus on MRI. Among these cases, some patients experience DPHL for approximately one month (4). DPHL due to CO poisoning is classically termed delayed neurological sequelae (5). The physiological mechanism of DPHL may involve myelin damage in the white matter. The hypothesis proposing the development of DPHL after a lucid interval is substantiated by factors such as the half-life of myelin (6).

HBO therapy is sometimes performed in patients with DPHL after CO poisoning; some studies have shown its efficacy (7). However, it remains unclear why HBO is effective in DPHL after CO poisoning. Furthermore, the equivalent effectiveness of HBO in DPHL from other DPHL causative agents has never been reported. Based on our experience in treating DPHL due to CO poisoning, a trial of HBO was performed, and significant effectiveness was observed. Concurrent with consecutive HBO therapy, the patient gradually improved the cognitive function and

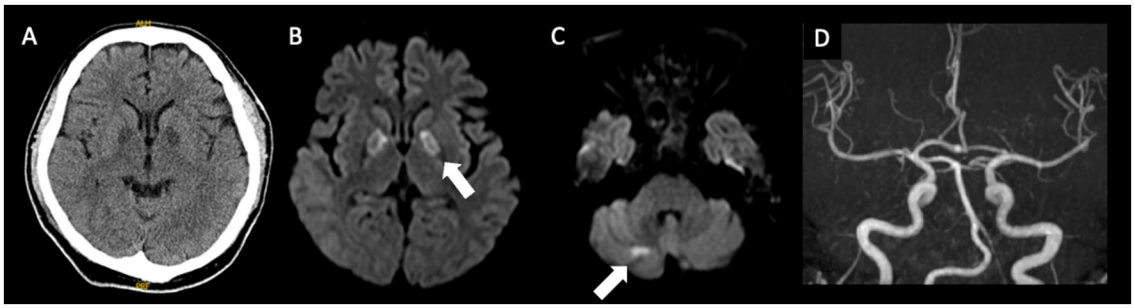

FIGURE 1
Imaging findings at the initial examination, **(A),** CT; **(B)**, **(C)**: MRI; **(D)**, MRA. Bilateral involvement of the globus pallidus and abnormalities in the right cerebellar hemisphere are evident. No major vessel stenosis is observed. CT, computed tomography; MRI, magnetic resonance imaging; MRA, magnetic resonance angiography.

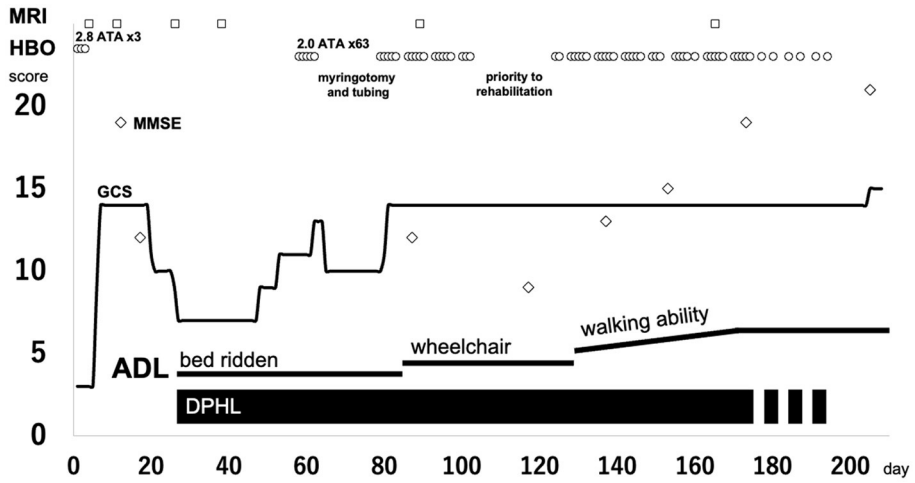

FIGURE 2
Clinical course of the patient. Circle, HBO; square, MRI taken; diamond, MMSE score. ADL, activities of daily living; DPHL, delayed post-hypoxic leukoencephalopathy; GCS, Glasgow Coma Scale; HBO, hyperbaric oxygen; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging.

Parkinsonism. However, determining whether HBO was practically effective in this case, whether the patient improved through natural course, or the extent to which HBO was necessary was difficult. Therefore, to verify the effectiveness of HBO, we discontinued the treatment after the 23rd HBO session, allowing the patient to concentrate on rehabilitation. However, the patient's symptoms stopped improving temporarily. Therefore, we resumed HBO after a while. HBO therapy was continued until recovery was validated using imaging studies.

White matter abnormalities observed on MRI gradually disappeared over time. The end of HBO therapy was determined by confirming significant improvement in the white matter lesions on MRI. However, follow-up ^{123}I-IMP-SPECT revealed residual damage in the frontal lobe. Severe DPHL cases often involve persistent frontal lobe dysfunction (2). The anatomically longer neuronal tracts in the frontal lobe may render it more susceptible to damage. Clinically, the patient remained incapacitated, reflecting persistent frontal brain dysfunction.

The value of using HBO for the treatment of mental illness has not been well-established (8). For example, the effect of HBO on post-traumatic stress disorder is controversial, with some negative (9) and some positive findings in the literature (10). However, recently, HBO has been reported to be effective in patients with post-stroke depression (11). Since there are some special cases, such as this case, continued research is needed to determine the neuroprotective effects of HBO under certain unique circumstances.

Unfortunately, neither DPHL nor CHANTER syndrome have been elucidated in detail due to their extreme rarity. Furthermore, the limited number of HBO facilities and its specialists has limited the discussion on these conditions. Based on our report, we recommend conducting further studies to confirm the effects of HBO treatment, as our report suggests a possible therapeutic effect of HBO on treating DPHL associated with opioid intoxicated CHANTER syndrome.

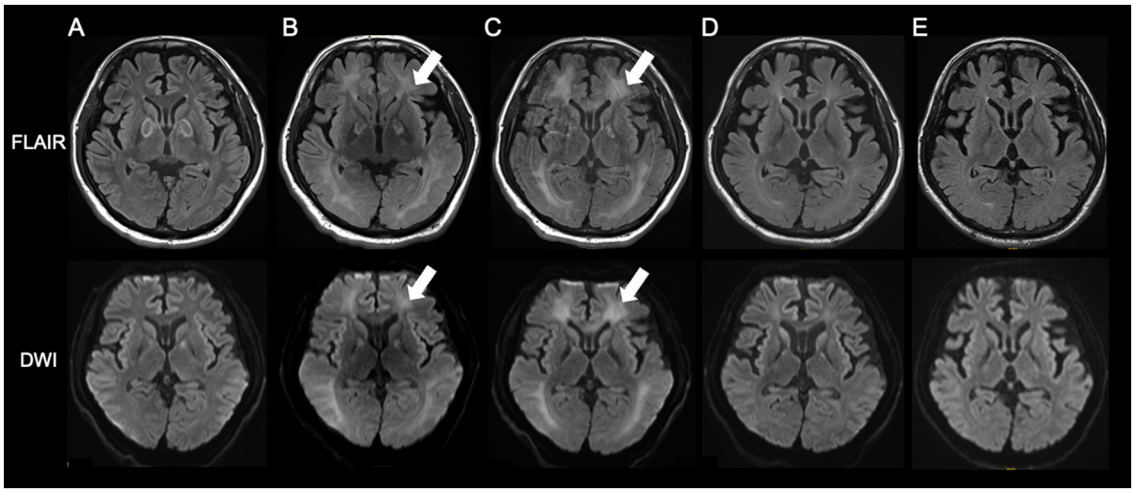

FIGURE 3
The course of MRI findings. **(A)** day 11; **(B)** day 25; **(C)** day 38; **(D)** day 89; **(E)** day 165. White matter abnormalities become apparent from day 25 and gradually disappear after consecutive HBO therapy. HBO, hyperbaric oxygen; MRI, magnetic resonance imaging.

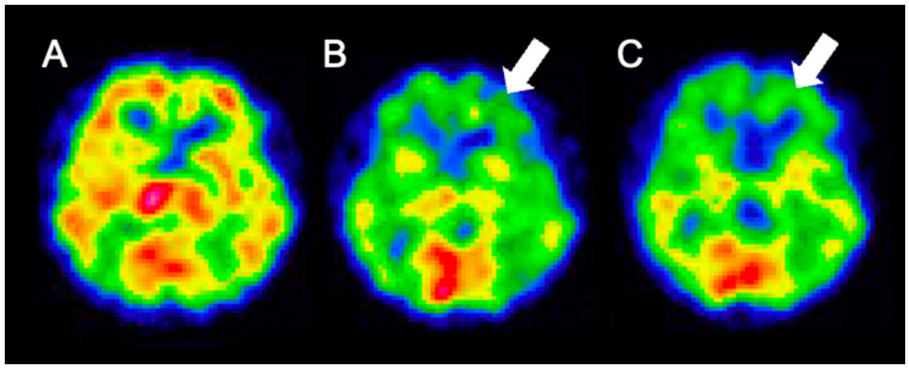

FIGURE 4
^{123}I-IMP SPECT findings. **(A)** day 12; **(B)** day 96; **(C)** day 164. The frontal lobe hypoperfusion is apparent on day 96 and persists. ^{123}I-IMP SPECT, N-isopropyl-p-[123I]- iodoamphetamine single-photon emission computed tomography.

In conclusion, the therapeutic effect of HBO is expected in patients with DPHL, even if the cause is not CO poisoning, as HBO enhances myelin regeneration by improving cerebral blood flow and oxygen supply.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

NJ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. KC: Data curation, Writing – review & editing. TN: Visualization, Writing – review & editing. MT: Data curation, Writing – review & editing. KK: Data curation, Writing – review & editing. RT: Data curation, Writing – review & editing. NS: Writing – review & editing, Data curation. SO: Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1364038/full#supplementary-material

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Systematic review and dosage analysis: hyperbaric oxygen therapy efficacy in the treatment of posttraumatic stress disorder

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Background: Studies of hyperbaric oxygen therapy (HBOT) treatment of mild traumatic brain injury persistent postconcussion syndrome in military and civilian subjects have shown simultaneous improvement in posttraumatic stress disorder (PTSD) or PTSD symptoms, suggesting that HBOT may be an effective treatment for PTSD. This is a systematic review and dosage analysis of HBOT treatment of patients with PTSD symptoms.

Methods: PubMed, CINAHL, and the Cochrane Systematic Review Database were searched from September 18 to November 23, 2023, for all adult clinical studies published in English on HBOT and PTSD. Randomized trials and studies with symptomatic outcomes were selected for final analysis and analyzed according to the dose of oxygen and barometric pressure on symptom outcomes. Outcome assessment was for statistically significant change and Reliable Change or Clinically Significant Change according to the National Center for PTSD Guidelines. Methodologic quality and bias were determined with the PEDro Scale.

Results: Eight studies were included, all with < 75 subjects/study, total 393 subjects: seven randomized trials and one imaging case-controlled study. Six studies were on military subjects, one on civilian and military subjects, and one on civilians. Subjects were 3-450 months post trauma. Statistically significant symptomatic improvements, as well as Reliable Change or Clinically Significant changes, were achieved for patients treated with 40-60 HBOTS over a wide range of pressures from 1.3 to 2.0 ATA. There was a linear dose-response relationship for increased symptomatic improvement with increasing cumulative oxygen dose from 1002 to 11,400 atmosphere-minutes of oxygen. The greater symptomatic response was accompanied by a greater and severe reversible exacerbation of emotional symptoms at the highest oxygen doses in 30-39% of subjects. Other side effects were transient and minor. In three studies the symptomatic improvements were associated with functional and anatomic brain imaging changes. All 7 randomized trials were found to be of good-highest quality by PEDro scale scoring.

Discussion: In multiple randomized and randomized controlled clinical trials HBOT demonstrated statistically significant symptomatic improvements, Reliable Changes, or Clinically Significant Changes in patients with PTSD symptoms or PTSD over a wide range of pressure and oxygen doses. The highest doses were associated with a severe reversible exacerbation of emotional symptoms in 30-39% of subjects. Symptomatic improvements were supported by correlative functional and microstructural imaging changes in PTSD-affected brain regions.

The imaging findings and hyperbaric oxygen therapy effects indicate that PTSD can no longer be considered strictly a psychiatric disease.

KEYWORDS

post traumatic stress disorder, hyperbaric oxygen therapy, treatment, trauma, anxiety disorder, PTSD, HBOT

Introduction

This is a systematic review of the evidence for hyperbaric oxygen therapy (HBOT) treatment of post-traumatic stress disorder (PTSD) symptoms.

Until recently, PTSD was implicitly a psychiatric disorder that ensues from a trauma that is sufficiently disturbing to leave a person with residual emotional and behavioral sequelae. The essential feature of PTSD, as defined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) of the American Psychiatric Association (1), is the development of characteristic symptoms following exposure to one or more traumatic events in which the individual is exposed to actual or threatened death, serious injury, or actual violence. Intrusive symptoms (nightmares and flashbacks) are the hallmark of PTSD, while avoidance, hypervigilance, cognitive and mood changes are seen in most cases. The symptoms, severity, and persistence vary widely for unknown reasons (2).

Epidemiological studies report that over half of the general population are exposed to a serious traumatic event in their lifetime, but only about 7 percent of those are likely to develop PTSD (3). However, among military personnel who have been involved in combat, the prevalence of PTSD is much higher and is often comorbid in 37% of servicemembers with mild traumatic brain injury (mTBI) (4).

Treatment of PTSD is problematic, especially for the persistent, disabling, or more severe forms. When PTSD becomes chronic nearly half of patients become treatment resistant (5, 6). Meta-analyses (7, 8) show that psychological interventions can significantly reduce PTSD symptoms when compared to control subjects; however, the effect sizes are often small or not reported. Pharmacological interventions were shown to be less effective than trauma-based psychological treatments for reducing PTSD symptoms or improving sleep with less than 60 percent reporting a meaningful clinical response and only 20 to 30 percent reporting remission (9, 10).

Improved brain imaging technology and research on U.S. war veterans from the Iraq and Afghanistan conflicts have revealed PTSD-associated changes in brain structure and function. This suggests that PTSD can no longer be considered a strictly psychiatric disease. Fear-conditioned learning was always identified as one of the main causative factors in PTSD. The growing literature, however, shows that PTSD is associated with specific neuroanatomical abnormalities or changes in fear neural pathways including the thalamus, amygdala, hippocampus, and medial prefrontal cortex (11–13). Veterans with severe PTSD show an indentation in the centromedial amygdala (14). A meta-analysis (15) reported a significant gray matter volume reduction of the left parahippocampal gyrus. Multiple studies demonstrate reductions in grey matter volumes in the amygdala, hippocampus, and prefrontal cortex, and the white matter volumes in the tracts connecting these three regions, including the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis (16). Studies also reveal that PTSD is associated with a smaller hippocampus (17, 18). People with PTSD have been shown to have decreased activity in the prefrontal cortex which helps regulate the emotional responses triggered by the amygdala. It is not clear whether some neural circuit PTSD-associated abnormalities are present before a trauma or if the abnormalities develop after. Nonetheless, exposure to traumatic events has been shown to cause long-term changes in brain activity and in microstructure (19–21). The PTSD-related brain changes identified in these studies suggest wounding and inflammatory processes (19) that could be responsive to treatments for wounds and inflammation (22).

Hyperbaric oxygen therapy (HBOT) has been historically defined as intermittent treatment with 100% oxygen at a minimum arbitrary pressure of 1.4 atmospheres absolute (ATA) and more recently 1.0 ATA for a narrow list of acute and chronic wound conditions (22, 23). The U.S. Food and Drug Administration (FDA) corrected the confusion and misunderstanding in this definition by re-categorizing HBOT as a prescription medical drug (oxygen) and device consisting of increased barometric pressure and hyperoxia (24). Scientifically, it has been defined as "a medical treatment that uses increased atmospheric pressure and increased oxygen as drugs by fully enclosing a person or animal in a pressure vessel and then adjusting the dose of the drugs to treat pathophysiologic processes of the diseases" (25, 26). The exposure to increased atmospheric pressure and hyperoxia must be intermittent to achieve the therapeutic benefit, but the length and depth of exposure, use, frequency, and number of air breaks, frequency and total number of treatments, total oxygen and pressure dose, i.e., all variables of dosing, have not been well-defined. Based on this scientific definition, HBOT can be appreciated as a treatment for common acute and chronic wound pathophysiology (23, 25, 27, 28) found in acute and chronic wound conditions (23, 25, 27–30) and inflammatory conditions (28–33).

PTSD responsiveness to HBOT was first suggested as a serendipitous finding in the treatment of a U.S. war veteran with mTBI Persistent Postconcussion Syndrome (PPCS) (34). This positive response was replicated in a case series of 30 mTBI PPCS veterans (87% with PTSD) (35, 36), and a series of U.S. Department of Defense (DoD) sponsored civilian (37) and military-conducted studies (38–41). Due to misinterpretation of the DoD studies' data based on design flaws and the confused definition of HBOT (22, 23), the efficacy of HBOT in mTBI PPCS as well as PTSD has been unclear. A recent systematic review and dosage analysis of these studies clarified the confusion (42). By analyzing both the combined and separate effects of increased barometric pressure and hyperoxia, according to the scientific and FDA's understanding of HBOT, efficacy for HBOT in mTBI PPCS was demonstrated in a narrow pressure and wide oxygen dose range. Using the same analysis, this is a systematic review of the effectiveness of HBOT in the treatment of both military individuals and civilians with PTSD symptoms or PTSD.

Methods

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (43). The PRISMA abstract and manuscript checklists are attached as Supplementary Files S1, S2.

Search method

PubMed, CINAHL, and the Cochrane Systematic Review Database were searched without filters or time limits from 9-18-2023 to November 23, 2023 for English language clinical articles with HBOT treatment and PTSD symptom outcome data, using the search terms "hyperbaric oxygen" or "hyperbaric oxygen therapy" AND "stress disorder," "posttraumatic stress disorder," "post-traumatic stress disorder," or "posttraumatic stress disorder, or "PTSD," or "PTSD symptoms," or "posttraumatic stress disorder symptoms." Inclusion criteria were studies reporting pre-and post-HBOT treatment data, adult subjects 18–65 years old, civilian, or military, with and without a history of mTBI. The searched lists from each database were crosschecked against each other by visual inspection of titles to remove duplicates. Reference lists were reviewed for additional studies. Pooled studies, case studies, long-term follow-up, or reviews were excluded. PTSD outcome data had to be: Posttraumatic Stress Disorder CheckList – Military (PCL-M), Posttraumatic Stress Disorder CheckList – Civilian (PCL-C), Posttraumatic Stress Disorder CheckList – 5 (PCL-5), PTSD Symptom Scale Interview (PSS-I), or the Clinician Administered PTSD Scale (CAPS or CAPS – 5). The search process consisted of first search (title screen), second search (abstract review of first search titles), third search (full-text articles of abstracts), fourth search (detailed review of full-text articles).

Demographics and data extraction

The demographic characteristics of the final selected articles are shown in Table 1. Numerical data includes date of publication, study design, number and sex of subjects, age, percentage diagnosed with PTSD, military or civilian, years of education and number of months from trauma to treatment with HBOT.

Data was extracted from each study and entered into Table 2. Numerical data include traditional pressure/oxygen dose parameters, length of time in each session, total number of HBOT's in the protocol, outcome measure used in each study, number of points change pre to post within each group, percent change in PTSD symptoms for the designated outcome instrument, *p*-value, and whether the number of points change meets the criteria for Reliable Change (RC) or Clinically Meaningful Change (CMC). The total summative oxygen dose in excess of room air at 1 ATA for a course of treatment in each group of every study was calculated in atmosphere-minutes (AMs) according to the formula:

$$\text{AMs} = \text{Hyperbaric Pressure}\left(\text{ATA}\right) \text{ x } \text{FiO}_2 \text{ x}$$
$$\text{Time of oxygen exposure}\left(\text{mins.}\right) \text{ x Number of HBOTs}$$

FiO_2 is fractional inspired oxygen percentage. The AMs were computed for every phase of a hyperbaric treatment: compression, time at depth, air breaks, and decompression. Constant compression and decompression rates were assumed. The average pressure from the surface to depth (compression) and depth to the surface (decompression) was multiplied by the FiO2 of the breathing gas for these phases. A linear increase in FiO2 and ~90% FiO2 was assumed by 8 min of compression (46) for protocols in monoplace (single-person) chambers that compressed with 100% oxygen.

The selected studies used different instruments with different scales to measure the same PTSD symptoms (8, 47). To normalize and account for the differences between instruments the PTSD symptom outcomes were converted to percent change from baseline pre-randomization/treatment. The percent changes were averaged for each dose of pressure and summative oxygen and compared separately by hyperbaric treatment pressure (ATA) and the summative oxygen dose (AMs). The amount of symptom change was assessed for statistical significance and whether the change was sufficient to be considered a Reliable Change (RC) or a Clinically Meaningful Change (CMC), according to the guidelines developed by the National Center for PTSD. PTSD symptom changes of 5 to 10 points are

TABLE 1 Demographics of the analyzed studies.

Study	Design	Number Ss, Sex (M, F, U)	Age (yrs.)	% Dx'ed. w/PTSD	Military(AD), Vet (V), Civilian (C)	Education (yrs.)	Time from trauma to HBOT (mos.)
Wolf et al. (38)	RCT, DB	50 (48 M, 2F)	28.3	U	MI-AD	12+	3–71
Cifu et al. (39)	RCT, DB	61 (M)	23.2	U	MI-AD	U	8.5
Miller et al. (40)	RCT, DB	72 (69 M, 3F)	31.4	65	MI-AD	2/3^{rds}'s with >12	22.9
Harch et al. (36)	Prospective Case Series	30 (28 M, 2F)	30.3	77	MI-(11 AD, 19 V)	13.1	40.2
Weaver et al. (41)	RCT, DB	71 (70 M, 1F)	32.8	49	MI-(68 AD, 3 V)	82% "some college"	25.6
Hadanny et al. (44)	RCT, SB C-O Control	30 (30F)	45.9	100	C	16.5	450
Harch et al. (37)	RCT, SB C-O Control	50 (21 M, 29F)	42.5	0	C-41, MI-9 (1-AD, 8-V)	14.0 (HBOT), 15.6 (Control)	55.2
Doenyas-Barak et al. (45)	RCT, SB	29 (U M, U F)	39 HBOT, 32C	100	MI-35 (all V)	14.2-HBOT, 13.7-C	138-HBOT, 133-C

RCT, randomized controlled trial; DB, double blinded; SB, single blinded; C-O Control, Cross-Over of the Control group to the Treatment; U, unstated, Military-AD, Military Active Duty; V, Veteran.

TABLE 2 Change in PTSD symptoms by study, pressure and oxygen dose.

Study/Type Ss[a]	ATA x O^2	Time/# HBOTs	O^2 Dose (AM)	Measure[b]	Pre-Post Tx Change	% Change/p[c]/ RC[d]
Wolf (38) / 1	2.4 × 100%	90 m / 30	6,900	1	50–41.6 = 8.4	−17% / 0.05 / RC
"	1.3 x air	90 m / 30	1,002	1	48.9–40.6 = 8.3	−17% / 0.05 / RC
Cifu (39) / 1	2.0 × 100%	60 m x 40	4,860	1	49.4–42.6 = 6.8	−14% / 0.05 / RC
"	2.0 × 75% (1.5)	60 m x 40	3,720	1	44.7–43.3 = 1.4	−3% / ns / ns
"	2.0 ×10.5%	60 m x 40	76	1	45.1–43.9 = 1.2	−3% / ns / ns
Miller (40) / 1	1.5 × 100%	50 m x 40	3,120	2	48.5–43.5 = 5	−10% / u / RC
"	1.2 x air	50 m x 40	600	2	53.5–42.1 = 11.4	−21% / u / CM
"	Routine Care	--	--	2	51.8–49.7 = 2.1	−4% / ns / ns
Harch (36) /1, 2	1.5 × 100%	50 m x 40	3,420	1, 3	63.4–46.8 = 16.6	−26% / 0.001 / CM
Weaver (41) / 1	1.5 × 100%	50 m x 40	3,120	2	54.9–45.6 = 9.3	−17% / u / RC
"	1.2 x air	50 m x 40	600	2	52.1–55.1 = +3	+6% / u / ns
Hadanny (44) / 4	2.0 × 100%	90 m x 60	11,400	3, 4, 6	28.9–20.7 = 8.2	−28% / 0.006 / u*
"	No Tx Control	--	--	3, 4, 6	29.9–28.4 = 1.5	−5% / ns / ns
"	2.0 × 100% C-O	90 m x 60	11,400	3, 4, 6	28.4–21.8 = 6.6	−23% / 0.005 / u*
Harch (37) /2,3,4	1.5 × 100%	50 m x 40	3,420	1, 2	37.9–26.0 = 11.9	−31% / 0.0001 / CM
"	No Tx Control	--	--	1, 2	39.7–37.5 = 2.2	−6% / ns / ns
"	1.5 × 100% C-O	50 m x 40	3,420	1, 2	39.7–29.2 = 10.5	−26% / 0.0001 / CM
D-Barak (45) / 2	2.0 × 100%	90 m x 60	11,400	5, 6	46.6–28.5 = 18.1	−39% / 0.001 / RC
"	Routine Care	--	--	5, 6	49.5–51.5 = +2.0	+4% / ns / ns

Negative values are a reduction in symptoms (improvement), positive values an increase (worsening) of symptoms.
[a]Type Subjects: 1 = Military Active Duty, 2 = Military Veterans, 3 = Civilians men, 4 = Civilians women.
[b]Measure 1 = PCL-M, Measure 2 = PCL-C, Measure 3 = SPECT, Measure 4 = PSS-I, Measure 5 = CAPS-5, Measure 6 = MRI, DTI, and fMRI.
[c]u = Unreported or Unknown information. ns = not significant.
[d]RC = Reliable Change; CM = Clinically Meaningful. Reliable change for CAPS-5 is >/= 13, For the PCL-M or PCL-C it is 5–10 and CM is >/= to 11 points. No Reliable Change information was found for the PSS-I but it is considered highly reliable and similar to the PCL.

TABLE 3 PEDro analysis of methodologic quality and risk of bias.

Items	1	2	3	4	5	6	7	8	9	10	11	Total
Wolf (38)	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	9
Cifu (39)	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	9
Miller (40)	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	8
Weaver (41)	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	9
Harch (37)	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	8
Hadanny (44)	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	8
D-Barak (45)	Y	Y	N	Y	N	N	Y	Y	Y	Y	Y	7

considered an RC for the PCL. A 10-to-20-point change is a CMC (48). A change in the CAPS is equivalent to a 0.75–0.82 SD change of PCL (47). A within-person CAPS-5 change of 12 or more points is an RC (47). CAPS changes were considered equivalent to PSS-I changes due to high internal consistency, high interrater reliability, and no difference between the measures (8). All searches, screening, selection, data extraction, and analyses were performed by the two authors independently and then together. No automation tools were used.

Methodologic quality and risk of bias assessment

The physiotherapy evidence database (PEDro) scale (49) was used to assess the methodologic quality/risk of bias of the randomized trials included in the final analysis and is presented in Table 3. Randomized trials are given a total score of 0–10 based on individual scoring of 10/11 items (the first, eligibility criteria, is omitted from the score because it is an external validity item), using 1 point for "present," and 0 points for "absent." The 10 items beginning with #2 are: (2) random allocation, (3) concealed allocation, (4) groups similar at baseline, (5) subject blinding, (6) therapist blinding, (7) assessor blinding, (8) 1 key outcome for >85% of subjects, (9) 1 key outcome: intention-to-treat analysis, (10) 1 key outcome between-group statistical comparison, and (11) 1 key outcome point measurements and variability. Scoring was performed by both authors based on stated scoring items in the text of each article and is presented in Table 3. Additional reporting bias and conflict of interest were noted for investigators who were employees of the funding source. All items were scored as 0 if they were not mentioned. Attempts were made to contact the authors of

the studies to resolve the scoring on allocation concealment. Quality of studies was judged according to the scoring legend of Cashin et al. (50): poor (<4), fair (4, 5), good (6–8), and excellent (9, 10). Studies were independently scored by one author, reviewed by the other author and then conjointly scored by both authors.

Results

Literature search yielded 115 articles [PRISMA flow chart (43), (Figure 1)]. Eight studies met inclusion criteria, were included in the final analysis, and are listed chronologically in Table 1. Four of the studies in Table 1, (38–41), were U.S. Department of Defense (DoD) sponsored. The subjects were active duty military or veterans from the Middle East conflicts with comorbid mTBI. Some percentage of all four studies' subjects had comorbid diagnoses of PTSD. All four studies were designed to compare the effects of hyperbaric oxygen therapy on mTBI. PTSD was a secondary measure using the PCL-M or PCL-C administered before and after treatment. All four studies also attempted to control the placebo effect of the hyperbaric exposure with purported sham exposures. These sham exposure groups were based on the historical misdefinition of HBOT (22, 23) that defined HBOT as the use of 100% oxygen at ≥1.4 ATA. The bioactive components of HBOT are increased barometric pressure and hyperoxia (25, 26, 42). A sham treatment must omit both of these to control for the independent effects of pressure and hyperoxia. By design none of the sham groups in the 4 DoD studies could do this. The sham exposures are pseudo-sham/pseudo-control groups that used lower doses of hyperbaric oxygen therapy as a treatment. Thus, they are analyzed as comparative dosing studies. The only DoD study with an acceptable control group (40) used a no-HBOT treatment control group, similar to the civilian studies (37, 44, 45).

Wolf et al. (38), the first of the DoD studies, is a comparative dosing study that randomized 50 active-duty military, to treatment

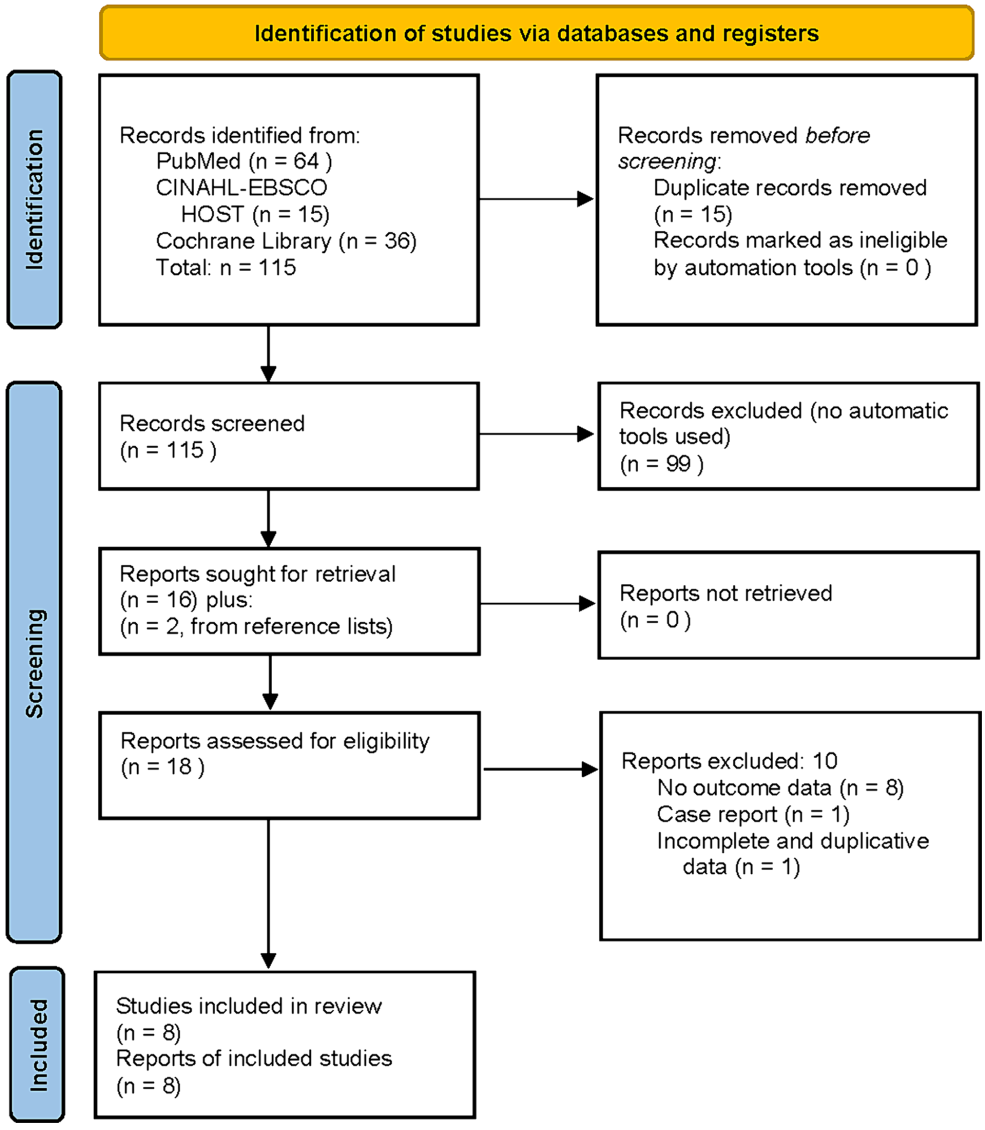

FIGURE 1
Literature search: hyperbaric oxygen therapy treatment of post-traumatic stress disorder.

and "control" groups for thirty 90-min exposures to 2.4 ATA 100% oxygen (2.4 ATA oxygen, 6,900 AMs oxygen) or 1.3 ATA 21% oxygen, room air (0.27 ATA oxygen, 1.002 AMs oxygen). Within-group change in PCL-M symptoms was a statistically significant 8.4-point RC decrease in PTSD symptoms for the 2.4 ATA group (50–41.6, $p < 0.05$) and a near identical significant RC decrease of 8.3 points (48.9–40.6, $p < 0.05$) for the 1.3 ATA 21% oxygen group. No statistical analysis of change scores between the two groups was performed. No conclusions were drawn about the efficacy of HBOT for PTSD.

Cifu et al. (39) was a similar comparative dosing study of three different doses of oxygen at a constant pressure. The study randomized sixty-one male military subjects to forty 60-min exposures to 2.0 ATA 100% oxygen (2.0 ATA oxygen, 4,860 AMs oxygen), 2.0 ATA 75% oxygen (1.5 ATA oxygen, 3,720 AMs), or 2.0 ATA 10.5% oxygen (0.21 ATA oxygen, 76 AMs). There were no differences in PCL-M scores between-groups before treatment or between groups after treatment, but no change score differences between-groups were analyzed. Within group analyses showed a statistically significant RC score PCL-M improvement of 6.8 points (49.4 to 42.6, $p < 0.05$) for the 2.0 ATA 100% oxygen dose group and non-significant change scores of 1.4 points (44.7 to 43.3) for the 1.5 ATA oxygen group and 1.2 points (45.1 to 43.9) for the 0.21 ATA oxygen group. No conclusions were drawn about the efficacy of HBOT for PTSD.

Miller et al. (40) is the only controlled DoD study. It randomized 72 subjects to forty 60-min exposures to 1.5 ATA 100% oxygen (1.5 ATA oxygen, 3,120 AMs), 1.2 ATA 21% oxygen, room air (0.25 ATA oxygen, 600 AMs), or a true control group of Routine Care without hyperbaric treatment. Within-group treatment effects showed a CMC 11.4 point PCL-C decrease (53.5–42.1) in the 1.2 ATA group, a 5.0 point RC decrease in the 1.5 ATA group (48.5–43.5), and a 2.1 point decrease (51.8–49.7) in the Routine Care group which was neither aCMC nor an RC. There were no between group, within group, or treatment vs. true control group statistical analyses performed. The study noted improvement in PTSD symptoms "after the interventions, favoring the "sham" group… over the HBO group…," but no conclusions were drawn about the efficacy of HBOT for PTSD.

The fourth DoD study, Weaver et al. (41) randomized 71 mostly active duty military members with mTBI PPCS to the same HBOT treatment schedule as Miller et al. (40), but did not include a true no-treatment control group. Subjects received forty 60 min exposures to 1.5 ATA 100% oxygen (1.5 ATA oxygen, 3,120 AMs) or 1.2 ATA 21% oxygen, room air (0.25 ATA oxygen, 600 AMs). Between-group analysis demonstrated a statistically significant change score difference between groups (improvement in favor of the 1.5 ATA oxygen group) of 7.3 points (−13.5, −1.0; $p = 0.02$), despite a significant disease severity bias against the 1.5 oxygen group. The 1.5 ATA oxygen group was older, had more combat deployments, worse anger control, and higher mean pre-treatment PCL-C scores than the 1.2 ATA group. When those subjects diagnosed with PTSD by the Structured Clinical Interview for DSM-IV PTSD module (the PTSD subgroups) were analyzed the 1.5 ATA oxygen HBOT benefit was even greater: a 12.3 point statistically significant (−21.4, −3.1; $p = 0.01$) difference between groups. This was composed of a 9.3 point RC improvement for the 1.5 ATA oxygen group and a 3.0 point worsening of the 1.2 ATA (0.25 ATA oxygen) group.

Summarizing the above four studies, RC improvements in PTSD symptoms have been demonstrated at 2.4 ATA oxygen and 1.3 ATA air (0.27 ATA oxygen) (38), 2.0 ATA oxygen (39), 1.5 ATA oxygen (40, 41), and CMC at 1.2 ATA air (0.25 ATA oxygen) (40). The Wolf et al. (38) and Cifu et al. (39) dose group improvements were statistically significant within-group changes and the Weaver et al. (41) study showed a statistically significant between group improvement in favor of the 1.5 ATA oxygen group compared to the 1.2 ATA air group. In the only study with a true control group, Miller et al. (40), the CMCs and RCs within-groups occurred only in the two treatment groups while none occurred in the No Treatment control group. The remainder of the within group changes (40), between group changes (38, 39), and changes compared to true control group (40) were not statistically analyed in the studies.

The remaining 4 studies consisted of a case series with imaging controls and three studies with true treatment controls. The U.S. DoD-administrated case series of Harch et al. (36) prospectively treated 30 active duty and retired military members with mTBI PPCS, 23 of whom met the PCL-M threshold for PTSD diagnosis. The subjects received forty 60-min exposures to 1.5 ATA 100% oxygen (1.5 ATA oxygen, 3,420 AMs oxygen). The subjects had the greatest pre-treatment PTSD scores (mean of 63.4) of all the studies with military subjects, which was 32% higher than subjects in the four DoD studies. These subjects experienced the greatest reduction in the PCL, 16.6 points. This was statistically significant (−22.6, −10.6; $p < 0.001$) and a CMC. The controlled neuroimaging component of the study demonstrated significant SPECT brain blood flow improvements in right posterior hemispheric gray and white matter regions in the HBOT-treated veterans compared to controls.

Two of the three remaining studies, Hadanny et al. (44) and Harch et al. (37), used a randomized crossover design where all subjects received HBOT and the randomly assigned control group crossed over to receive HBOT after the control period. Hadanny et al. (44) is the only study that was exclusively non-military subjects, all women (with sexual trauma in childhood), and had no comorbid TBI. Thirty women received sixty 90-min exposures to 2.0 ATA 100% oxygen (2.0 ATA oxygen, 11,400 AMs). The treatment group experienced a statistically significant 8.2 point (28.9–20.7, $p = 0.006$) decrease in the PSS-I compared to 1.5 points in the control group (29.9–28.4, $p = 0.22$). After the control period the control group crossed-over and was treated with the same 2.0 ATA 100% oxygen, 11,400 AMs. Following treatment, the crossed-over control group experienced a significant 6.6 point (28.4 to 21.8, $p = 0.005$) decrease in PTSD symptoms. No statistical analysis of change scores between groups was performed. The raw point reductions would be reliable changes on a PCL questionnaire. Considering the condensed PSS-I scale vs. the PCLs (51 points vs. 85 points) if re-scaled to a PCL questionnaire the scores for CMC and RC would be 14.5 and 11.0 points, respectively. SPECT brian blood flow imaging analysis revealed significant increases in blood flow in 7 Brodmann areas compared to the control group. Correlation analysis of perfusion changes and questionnaire scores were significant between 7 Brodmann areas and multiple questionnaire components. MRI DTI results demonstrated significant increases in fractional anisotropy (FA) in anterior thalamic radiation, left insula, right thalamus, and superior thalamic radiation ($p < 0.001$) in HBOT subjects compared to controls. When all HBOT-treated subjects (HBOT group and crossover Control Group) were compared to Controls pre-HBOT three of the four significant findings persisted; superior thalamic radiation was no longer significant.

The second crossover study, Harch et al. (37), was a study on mTBI PPCS that excluded subjects with CAPS scores high enough to

be diagnosed as PTSD. Sixty-three subjects, 83% civilian and 17% military, were randomized to forty 60-min exposures to 1.5 ATA 100% oxygen (1.5 ATA oxygen, 3,420 AMs) or a true control group without hyperbaric treatment. The Control group then crossed over to receive the same 1.5 ATA oxygen HBOT treatment at the end of the control period. Between-group analysis of PCL change scores demonstrated a statistically significant change score difference (improvement in favor of the 1.5 ATA oxygen group) of 9.7 points (−17.7, −8.6; $p = 0.0001$). Within-group analyses showed a statistically and CMC score improvement of 11.9 points (37.9–26.0, $p < 0.0001$) for the 1.5 ATA oxygen dose group and non-significant change scores of 2.2 points (39.7–37.5, p = n.s.) for the no treatment control group. After the Controls crossed over and received HBOT treatment they achieved a similar statistically significant and CMC decrease in PCL-C of 10.5 points (37.5–27.0, $p < 0.0001$).

The final study by Doenyas-Barak et al. (45) was similar to Hadanny et al. (44). Both studied treatment-resistant PTSD without comorbid TBI using identical doses of HBOT, but Doenyas-Barak et al. (45) studied military members in a treatment vs. true control no-treatment design, while Hadanny et al. (44) studied civilians in a crossover true control design. Doenyas-Barak et al. (45) randomized 28 veterans to sixty 90-min exposures to 2.0 ATA 100% oxygen (2.0 ATA oxygen, 11,400 AMs) or a control group without HBOT treatment who received their ongoing psychotherapy. Within-group analyses demonstrated a statistically and clinically significant decrease (improvement) in CAPS-5 score of 18.1 points in the HBOT group (46.6–28.5, C.I: −25.4, −10.8, $p < 0.0001$) compared to a 2.0 point increase (worsening) in the control group (49.5–51.5, $p = 0.211$). Cohen's net effect size was 1.643. Translating the CAPS to a PCL score indicates that this is a 25.9 point decrease in the PCL, a very large change score. Brain imaging MRI DTI demonstrated significant increases in fractional anisotropy in the left anterior and posterior limbs of the internal capsule and right parietal white matter in HBOT-treated subjects compared to controls. Functional MRI showed within-group increases in fMRI BOLD signal for the HBOT-treated subjects in the left dorso-lateral prefrontal, middle temporal and temporal gyri, both thalami, left hippocampus, and left insula. No significant within group changes were found for the control group. Statistically significant correlations were found between mean percent BOLD signal changes in peak significantly activated regions and percent change in total CAPS score (r = 0.42–0.67, $p < 0.05$).

In summary, six of the eight studies were conducted on military subjects and two on civilians. Two studies were designed to investigate PTSD without TBI. One of these studies was on military subjects with treatment resistant PTSD who did not have a TBI and the other involved women who were sexually abused as children with long-term PTSD. There are four studies with five pseudo-sham control groups that received lower doses of hyperbaric oxygen therapy. Two of the five pseudo-sham groups showed significant improvement in PTSD, one at 1.3 ATA and the other at 1.2 ATA. The other 3 pseudo-sham treatments only showed −1 point (improvement) to +3 points (deterioration).

Dose analysis of studies

Percent change in immediate post-treatment or control PTSD symptoms for each study's groups is presented in Table 2. These percent changes are further compared by treatment pressure (Figure 2) and total oxygen dose from the repetitive intermittent exposures to hyperoxia (Figure 3). Figure 2 shows a minimum pressure threshold for improvement in PTSD symptoms at 1.3 ATA that is relatively constant for increased pressures upto 2.4 ATA. Figure 3 shows a minimum total oxygen dose threshold for improvement in PTSD symptoms of 1,002 AMs (20% improvement) and varied improvements in symptoms with greater oxygen doses upto 11,400

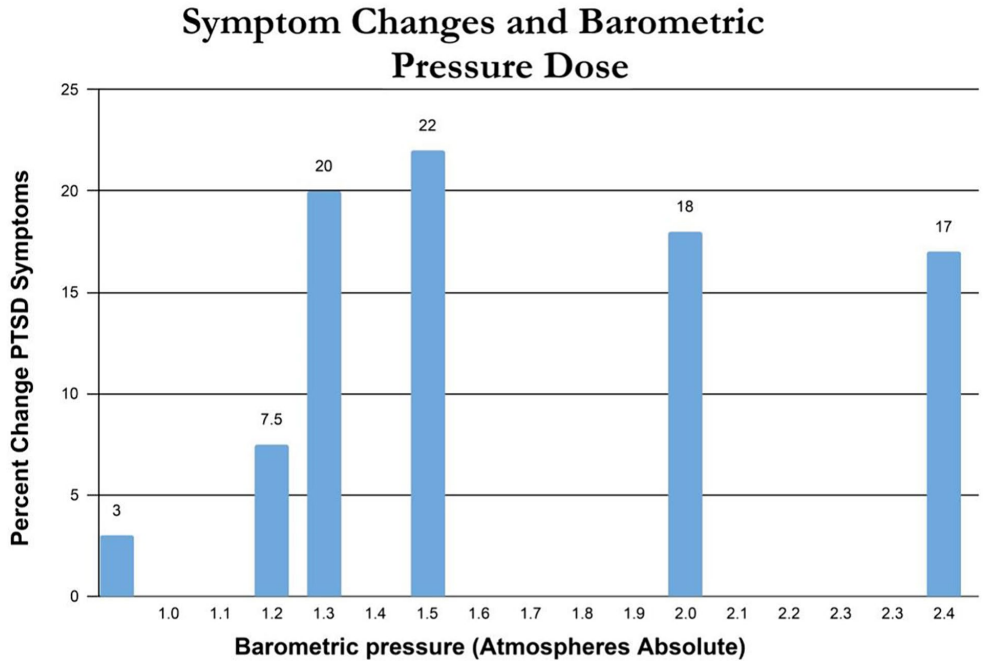

FIGURE 2
Symptom changes and barometric pressure dose.

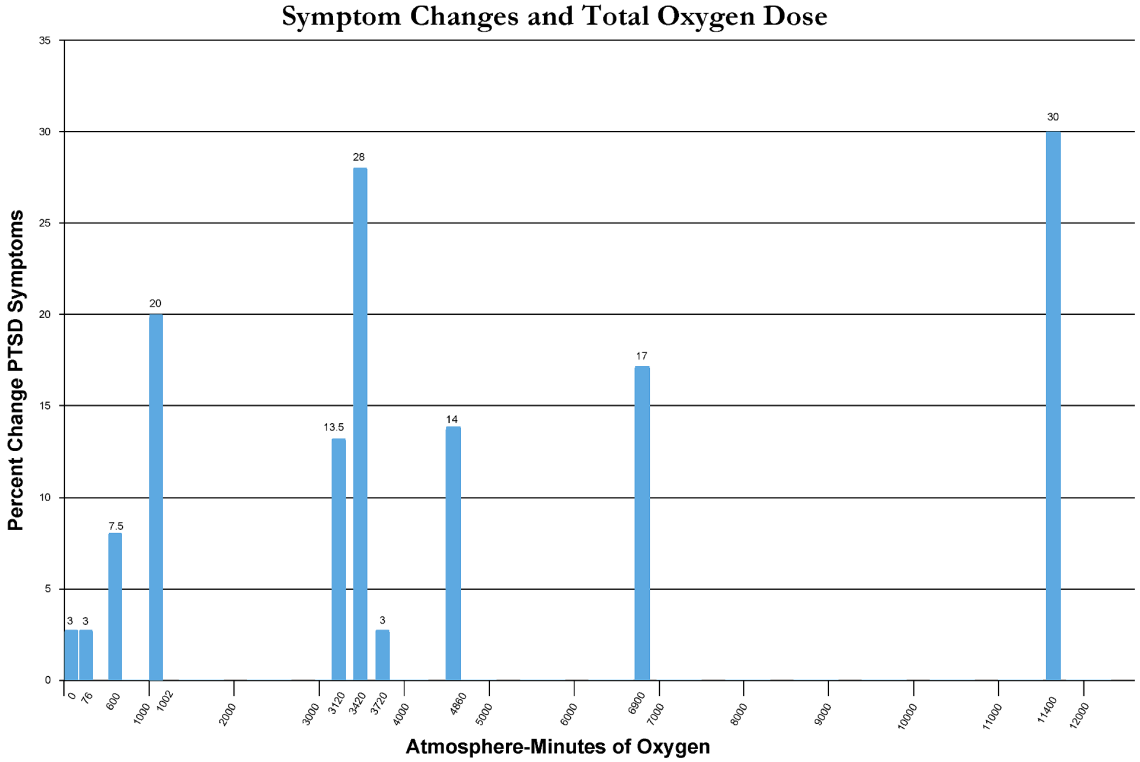

FIGURE 3
Symptom changes and total oxygen Dose.

AMs (13.5–30%). The one outlier figure of 3,720 AMs (3% improvement) in this range of AMs greater than 1,002 AMS was the only mixed dose of oxygen and pressure where oxygen was not 100% of the total pressure dose (1.5 ATA oxygen, 2 ATA pressure).

To clarify the effect of total oxygen dose on outcome from intermittent exposures to hyperoxia Figure 3 outcomes were graphed with average % improvement in PTSD symptoms for narrow ranges of total oxygen dose (Figure 4). Figure 4 demonstrates a near-linear dose–response curve for increasing PTSD symptom improvement with increasing total oxygen dose.

Methodologic quality, and risk of bias assessment

According to PEDro scoring statistics (51), studies scoring ≥6/10 on the PEDro scale are considered "moderate to high quality." Table 3 presents the PEDro analysis for the seven randomized studies reviewed. All seven scored studies met criteria for moderate to high quality. Using the qualitative assessment of Cashin et al. (50) for the entire scale of PEDro Scale scores, all four randomized controlled trials (37, 40, 44, 45) and all three randomized non-controlled studies were considered good to excellent quality/bias with PEDro scores of 8 (40), 8 (49), 8 (37), 9 (38), 9 (39), 9 (41), and 7 (50). Despite the methodological rigor of these hyperbaric studies, PEDro scores are compromised by 1–2 points each due to the inherent blinding problems of hyperbaric treatment, similar to surgical and other human performed therapies. Chamber operators (therapists) cannot be blinded to treatment group in any of the studies (1 point) and subjects are not blinded in any no-treatment control group study (1 point), especially the crossover studies. An additional source of bias not addressed in the PEDro analysis is bias from conflict of interest. Dr. Harch stated conflict of interest in his studies (36, 37). Many of the investigators in the other studies are employed by the funding source (38–41, 45) or have another conflict of interest (51).

Side effects

Side effects and adverse events are listed in Table 4 and were generally minor and transient. Mild reversible middle ear barotrauma (MEBT), the most common complication seen in HBOT studies, occurred in 5.5–43% of the subjects. This range was higher than historical figures of 2% (23), the incidence in Harch et al. (37). Some of the higher figures were explained by chamber operations/operators/equipment at one study site (41) and intensity of schedule (twice/day treatments) with more frequent upper respiratory infections (36). The high rate was not explained in the other studies (44, 45). Total side effects and adverse events were combined and reported in Churchill, et al. (52) for Weaver et al. (41) and Miller et al. (40) at 33% (40/120), 77% of which were due to otic, sinus, and tooth barotrauma. Total complication rate in Harch et al. (37) was 8%.

Three of the studies reported an unusual side effect, worsening of emotional symptoms transiently during treatment that occurred in at least 30% of subjects. Harch et al. (37) noted exacerbation of PTSD anxiety (2/30 subjects, 6.7%), and transient worsening of some TBI/PTSD symptoms at the midway point (7/30 subjects, 23%), while Hadanny et al. (44) documented 32% (9/28 subjects) with "emotional flooding" (re-experiencing memories of childhood event) during the

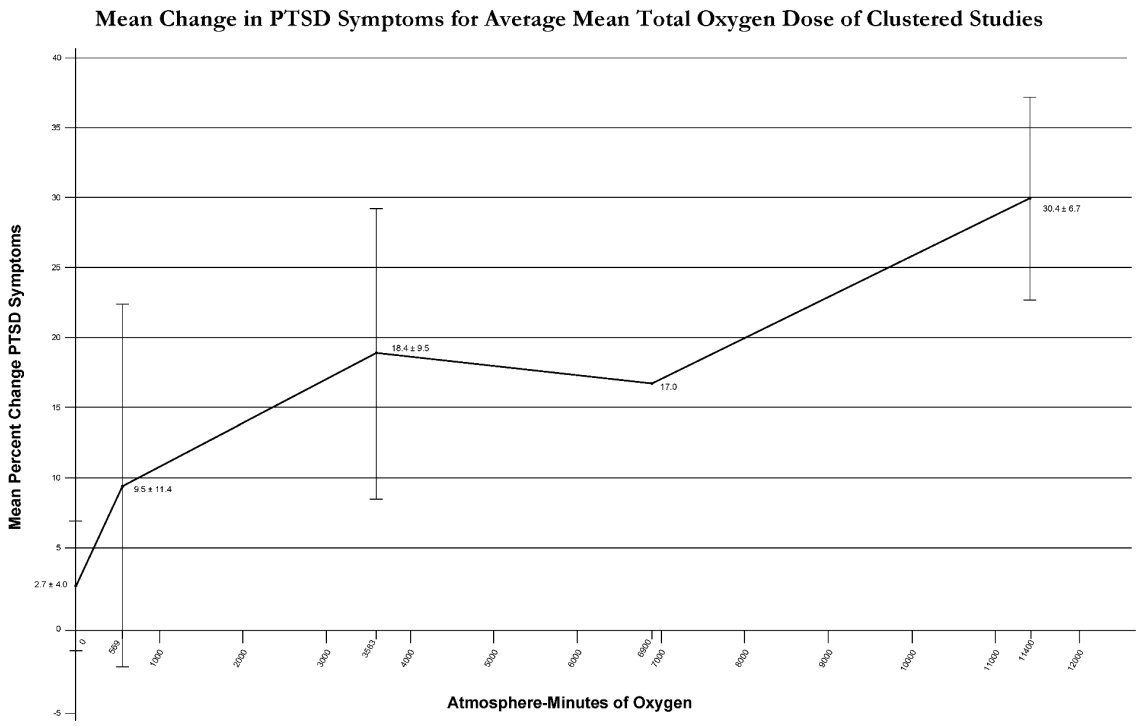

FIGURE 4
Mean change in PTSD Symptoms for average mean total oxygen dose of clustered studies.

TABLE 4 Side effects and complications.

Study	Middle ear barotrauma	Other minor side effects	Significant adverse events
Wolf (38)	5.5%	0.07–0.61%: sinus squeeze, confinement anxiety, headache, nausea, numbness, heartburn, musculoskeletal chest pain, latex allergy, and hypertension	
Cifu (39)	None reported	None reported	None reported
Miller (40)	Reported combined with Weaver, *et al*	Reported combined with Weaver, *et al*	Reported combined with Weaver, *et al*
Harch (36)	20%	23%: transient worsening of TBI/PTSD symptoms at midway through treatment	6.7%: transient exacerbation of anxiety
Weaver (41)	17%	8.3%: sinus barotrauma 0.8%: tooth barotrauma. 6.7%: headache, 2.5%:dizziness/vertigo 2.5%: vision change, 1.6% each: anxiety and somnolence, 0.8% each: dyspnea, neck irritation, eye pruritis, or hyperventilation	
Hadanny (44)	43%	3.6%: headache	32%: reversible emotional flooding during 1st 20 HBOTs
Harch (37)	2%	1%: perforated tympanic membrane, 4%: late protocol fatigue	
Doenyas-Barak (45)	39%		39%: reversible surfacing of new memories/severe distress

first 20 HBOTs, and Doenyas-Barak et al. (45) reported a similar 39% (7/18 subjects) with "unexpected surfacing of new memories…" that "surfaced gradually, during the second half of the treatment course (after 25–35 sessions of HBOT)." Some of these three studies' events appear to be serious adverse events. Two of Harch et al.'s (37) patients required emergency department visits, one of which re-instituted

benzodiazepine medication that had been discontinued before the event that occurred during alcohol intoxication, and the second for evaluation of anxiety-induced chest pain/gastrointestinal distress. Hadanny et al. (44) does not elaborate on the severity of the emotional flooding. Doenyas-Barak et al. (45) stated that the recovery of the memories was accompanied by severe distress with "…resolution of the distress." Despite this exacerbation of emotional symptoms in the studies all of the subjects were able to complete the treatment.

Discussion

In this systematic review of HBOT treatment of PTSD symptoms eight studies (7 randomized trials) were analyzed for symptom outcomes according to composite doses of hyperbaric oxygen therapy and component doses of barometric pressure and hyperoxia. Overall, the studies' data demonstrate that HBOT caused statistically significant, reliable change, or clinically meaningful change/improvements in PTSD symptoms and/or PTSD compared to true control groups and within nearly all treatment groups. In two of the studies with the greatest improvement symptom relief was obtained for treatment-resistant PTSD that was over a decade long. The improvements in three of the studies were supported by functional and anatomic imaging changes in PTSD-associated brain structures. These results were achieved despite heterogeneity of study design, statistical analysis, and subject populations. Treatment effects were non-specific to cause of PTSD, occurring in male and female subjects, both military and civilian, with childhood sexual trauma and fibromyalgia.

The symptom improvements were achieved with multiple different doses of HBOT and occurred at composite doses of oxygen and pressure at 1.3, 1.5, 2.0, and 2.4 atmospheres. The pressure threshold for improvement appeared to be 1.2–1.3 ATA and the total oxygen threshold of 1,002 atmosphere minutes. There was increasing benefit with increasing total doses of oxygen from 1,002 to 11,400 AMs. These total doses of oxygen are summative from 30 to 60 repetitive intermittent exposures to hyperoxia and pressure. The average greatest symptom improvement occurred with the highest total oxygen dose in two studies (44, 45), but the dominant contributing component(s) to this dose (FiO2, absolute pressure, length of each treatment, absence/presence/number of air breaks, treatment frequency, and total number of treatments) could not be identified. It may have been the presence of all of these variables in the regimen of these two studies that is responsible for the large symptom improvement, however a near equivalent percent symptom improvement occurred in Harch et al. (36) in similar subjects (44) with the same FiO2, half the increase in barometric pressure, half the treatment length, no air breaks, twice the frequency, two-thirds the number of treatments, and with a lesser incidence of emotional flooding/side effects. Due to the small number of studies and large number of variables it is impossible to draw further conclusions beyond increasing symptom improvement with increasing total oxygen dose. There is no evidence, however, that the HBOT-induced effects on PTSD could occur from a continuous total exposure to oxygen of 1,002–11,400 AMs.

All seven randomized trials were judged to be of moderate to high quality by the PEDro scoring system. The rigor of all seven studies was excellent, but due to limitations of blinding inherent to hyperbaric oxygen treatment their PEDro bias ratings were compromised by at least one point and in four of the seven by two points. PEDro criteria require blinding of subjects and all "therapists." To blind subjects to both pressure and oxygen they must not go in a hyperbaric chamber. A no-chamber experience group unblinds the control subjects [one point PEDro scale loss each for four of the studies (37, 40, 44, 45)]. To blind all therapists, which includes chamber operators/gas composition allocators, the operators/allocators cannot know the pressures or gas compositions. Using the most sophisticated chamber designs and equipment, this is nearly impossible to achieve and was not achieved in the DoD studies (38–41): one-point PEDro scale loss each.

The inability to blind subjects and operator therapists to treatment group should predispose to placebo effects that lower the rigor and increase the bias of a study. It appears that these PEDro scale losses did not compromise the rigor of the studies because the placebo effect in these studies was negated by the pressure and oxygen dose analyses. The analyses showed that there are minimum thresholds necessary for treatment effects. If placebo was involved the treatment effect should occur regardless of oxygen or pressure dose. That did not occur. In addition, different effects were seen with different doses which is contrary to a placebo contribution. The placebo contribution is also negated by the Cifu et al. study (39) in which all subjects had the identical chamber experience yet only one group showed a significant outcome. If the ritual of the chamber experience had a placebo effect all of Cifu et al.'s (39) groups should demonstrate the same placebo outcome. They did not. Essentially, the PEDro scale overestimated the bias in the studies.

The most common side effect seen in HBOT, middle ear barotrauma, occurred in 5.5–43% of subjects. The higher range of this side effect in some of the studies was due to intensity of schedule in one study (37) and chamber operations in another study (41). In two other studies with the highest rates (44, 45) this was not explained. Other side effects were minor and/or rare. An unusual side effect that was documented in several studies was a significant, but reversible, revisiting of the old traumatic memories at the highest oxygen doses in 30–39% of subjects (44, 45). The surfacing of old traumatic memories is also considered to be part of the emotional healing process of PTSD, particularly when psychotherapy is provided at the same time. The investigators in these two studies suggest revisiting these memories is due to HBOT-induced metabolic and circuitry changes in brain areas associated with emotional and pain processing (44) and induced neuroplasticity effects in the hippocampus (45). Alternatively, this phenomenon may partly reflect increasing oxidative stress. The resurfacing of old memories was minor in the lower oxygen dose studies (36, 37), but more intense with the highest doses of oxygen, suggesting accumulating oxidative stress/toxicity from the intermittent exposure (42), despite air breaks. In the military study of Doenyas-Barak et al. (45) it occurred gradually with increasing number of treatments >25–35 HBOTs (2.0/90): $2 \times 90 \times 35 = 6{,}300$ AMs, which is the similar point at which reversal of improvement in PTSD and mTBI PPCS symptoms occurred in the military study of Wolf et al. (38): 30 HBOTs at 2.4/90: $2.4 \times 90 \times 30 = 6{,}480$ AMs. The Wolf et al. (38) effect at 2.4 ATA of oxygen has been identified as an oxidative stress/toxicity phenomenon (42). Regardless of the intensity, these side effects resolved during the completion of the therapeutic process.

PTSD is a mental health condition triggered by a terrifying event that causes flashbacks, nightmares, and severe anxiety. Once considered primarily a psychiatric disorder, PTSD is now understood to be accompanied by significant changes in the brain (11–21). These neuroanatomical changes help explain the behavioral changes and symptoms as the brain controls our behavior. However, more than

explaining the behavioral changes of a person suffering from moderate to severe PTSD symptoms, the brain changes may also help us understand why almost 50% of PTSD sufferers fail traditional treatments and their PTSD becomes chronic and resistant to treatment. Many studies have demonstrated the brain changes in PTSD subjects (11–21); however, this is the first review of studies that measured pre-and-post HBOT brain changes.

Three of the studies in this systematic review (35, 36, 44, 45) included neuroimaging to measure brain changes in chronic and/or treatment resistant subjects as a function of the HBOT treatment. Harch et al. (36) demonstrated significantly increased SPECT brain blood flow in the right posterior hemispheric gray and white matter areas in the HBOT treated veterans compared to controls. Hadanny et al. (44) also included SPECT imaging and the brain blood flow imaging analysis shows significant increases in blood flow in 7 Brodmann areas compared to the control group before cross-over. Hadanny et al. (44) reported MRI DTI results of significant increases in FA in anterior thalamic radiation, left insula, and right thalamus compared to the control subjects pre-cross-over. The significant increases in FA persisted after all control subjects were treated. It is relevant that these women had chronic PTSD that went back to their childhood abuse, but none of the subjects had a history of brain trauma, such as mTBI.

The third study, Doenyas-Barak et al. (45), also studied treatment-resistant PTSD in military veterans with no history of TBI. The study included MRI DTI and demonstrated statistically significant increases in FA in the left anterior and posterior limbs of the internal capsule and right parietal white matter in subjects who received HBOT treatment versus the control subjects. Subjects also showed within-group increases in the fMRI BOLD, which signals an over-oxygenation (actively actuated increase in blood flow and volume) (53) for the HBOT subjects in the left dorso-lateral prefrontal, middle temporal, and temporal gyri, both thalami, left hippocampus and left insula. The increases in fMRI BOLD are usually regarded as increased/improved activity due to flow-metabolism coupling. No within group changes were found for the No Treatment Control subjects.

Hyperbaric oxygen therapy is a dual-component drug therapy consisting of intermittent increased barometric pressure and hyperoxia (25, 26) that has wide-ranging beneficial effects on acute and chronic wound pathophysiology (23, 25, 27, 28) found in acute and chronic wound conditions (23, 25, 27–30) and inflammatory conditions (28–33). HBOT has been demonstrated to have wide-ranging effects on inflammation and a dysregulated immune system (54–59) that may be due to its broad effects on expression and suppression of immune-active genes (60–62). The anatomic and functional imaging findings (11–21) and immune dysregulation in PTSD (19, 63) imply that the experience of a terrifying traumatic event triggers wounding and inflammatory changes in the brain. The structural and functional imaging changes documented in the reviewed studies (35, 36, 44, 45) suggest that the HBOT-generated improvement in PTSD symptoms are the result of HBOT's well-documented effects on wound pathophysiology and wound-healing. This is apparent in the improvements in connectivity and blood flow in the hippocampus documented by Harch et al. (35) and Doenyas-Barak (45). Given the known immune system-activity of HBOT it is also likely that the symptomatic improvements are in part due to HBOT's beneficial impact on the immune system dysregulation in PTSD. These imaging and immune system findings coupled with HBOT's effects on inflammation and wounds suggests that PTSD can no longer be considered strictly a psychiatric condition.

Summary of main findings

This systematic review demonstrated statistically significant, clinically meaningful, or reliable change improvements in multiple high quality low bias randomized controlled and randomized trials of HBOT treatment of subjects with PTSD or PTSD symptoms. Minimum thresholds of pressure and total hyperoxic dose were required for the improvements, but occurred across a broad range of pressures and total oxygen doses with a dose–response relationship of symptom relief to total oxygen dose. The greatest symptom relief was achieved at the highest doses, but were accompanied by a side effect of severe emotional distress in upto 39% of subjects that was reversible. Based on these studies' data and this systematic review HBOT should be recommended as a treatment option for PTSD or patients with PTSD symptoms.

Limitations

Possible minor limitations include the limited number of studies, PTSD as a secondary outcome in many of the studies, and the majority of the studies in military subjects. The minimal bias of the studies and uniformity of results within the structure of a systematic review, according to pressure and oxygen dose and regardless of military or civilian status, cause of PTSD, or sex of subjects, suggests that these are not significant limitations. The apparent major limitation of this review is the heterogeneity of the studies: designs, doses of hyperbaric therapy, dosing parameters, subjects, diagnoses (PTSD symptoms or PTSD, with or without comorbid TBI or fibromyalgia), and statistical analyses. Some were randomized trials comparing different doses of hyperbaric therapy, some had true no-treatment control groups, there was a wide range of pressures and total oxygen doses, presence or absence of air breaks, many of the studies consisted of predominantly males, one was all female, and some studies analyzed only within group treatment effects while others looked at pre-and post-treatment effects or between group change effects. This apparent limitation could be interpreted as a strength of the study and its conclusions. The PTSD population is a heterogenous population and the majority of the studies in this review involved comorbid mTBI PPCS patients which are a very heterogeneous group by nature of their comorbid diagnosis. This would normally argue for very large studies to neutralize the effects of so many variables. However, each of the limitations applied to only some of the studies and were balanced by similar treatment results in other studies without the limitations. Each of the limitations would be expected to compromise uniformity of results, but despite the heterogeneity the improvement in symptoms was substantial and when analyzed by dose shown to be fairly uniform above certain pressure and total oxygen thresholds, contrary to placebo effects.

Another apparent traditional limitation is the small sample size of the studies. Large, randomized trials are preferred for systematic reviews and evidence grading, however, small sample size as a contributor to a Type I error is often the result of imbalance of treatment groups for key variables that affect the outcomes. None of the studies featured unbalanced groups except Weaver et al. (41)

where there was bias against treatment effect in the more severely affected HBOT group. Multiple Type I errors to explain multiple small trial positive results is unlikely. In addition, small sample size is usually a criticism of studies with negative outcomes due to a Type II error. The analyzed studies had positive outcomes. Overcoming a small sample size Type II error occurs when the treatment effect is large. The treatment effect was large in nearly all of the studies with CMC and RC. This supports the positive outcomes and conclusions of this systematic review.

Implications for future research

This systematic review sheds new light on PTSD and its treatment. It implies that HBOT may be the treatment of choice when pathological brain changes exist, such as in two of the studies in this review with complex treatment-resistant PTSD (44, 45). New research could focus on identifying those PTSD patients with pathological brain changes and which elements of the trauma-PTSD pathogenesis predispose to brain pathological changes: the intensity or duration of the trauma experience, the duration of the PTSD, treatment resistance, or other factors. It is possible that milder or shorter-term reactions to trauma (PTSD symptoms without the diagnosis of PTSD) may not have permanent brain changes, however, it appeared from the Harch et al. RCT (37) where the diagnosis of PTSD was excluded that milder or shorter-term reactions to trauma may also be responsive to HBOT. Future research should be directed to comparative effectiveness studies of HBOT versus standard therapies in these milder cases. Another research focus would be the identification of biomarkers or behavioral markers of pathological brain changes that would avoid costly neuroimaging. A possible candidate is microRNA. MicroRNA has been successfully investigated in acute mTBI with (64) or without (65, 66) balance, cognitive testing, and symptoms.

Conclusion

Multiple high quality low bias randomized controlled trials and randomized trials of HBOT in the treatment of PTSD symptoms or PTSD demonstrated statistically significant, clinically significant, and/or reliable change improvements in symptoms that occurred across a wide range of oxygen and pressure doses with a dose–response relationship for symptom relief and total oxygen dose. Side effects were minor except for a transient period of significant emotional symptoms at the highest oxygen doses.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. PH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

SA is self-employed in a private practice of neuropsychology. PH is employed by Harch Hyperbarics, Inc.

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Case report: Dementia sensitivity to altitude changes and effective treatment with hyperbaric air and glutathione precursors

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A 78-year-old man with dementia experienced waxing and waning of symptoms with changes in altitude as he traveled from his home in the Rocky Mountains to lower elevations and back. To replicate the improvement in his symptoms with travel to lower elevations (higher pressure), the patient was treated with a near-identical repressurization in a hyperbaric chamber using compressed air. With four 1-h treatments at 1.3 Atmospheres Absolute (ATA) and concurrent administration of low-dose oral glutathione amino acid precursors, he recovered speech and showed improvement in activities of daily living. Regional broadcast media had documented his novel recovery. Nosocomial COVID-19 and withdrawal of hyperbaric air therapy led to patient demise 7 months after initiation of treatment. It is theorized that hyperbaric air therapy stimulated mitochondrial biochemical and physical changes, which led to clinical improvement.

KEYWORDS

hyperbaric, glutathione, pre-cursors, altitude, wellness, pandemic, dementia, COVID-19

Introduction

The effects of barometric pressure on human physiology and cognition date back to Evangelista Torricelli's invention of the barometer in 1643. In 1644, Torricelli observed, "We live submerged at the bottom of an ocean of the element air."(1) Altitude ascents and descents in air have an effect on human physiology due to changes in both oxygen pressure and barometric pressure. The positive effects of descent from altitude on human pulmonary, rheumatic, and cardiac diseases have been documented during relocations of people from the Rocky Mountains (5000–10,000 ft above sea level) to the lower Missouri River Basin of the United States (~1000 ft above sea level) (2, 3). Kramer et al. (4) quantified and showed the durability of this phenomenon in chronic obstructive pulmonary disease (COPD) patients who relocated for 3 weeks from Jerusalem (altitude 800 m, 2500 ft, 13.4 psi, 0.91 ATA) to the Dead Sea (altitude 402 m of air below sea level 15.4 psi, 1.05 ATA). At the end of the Dead Sea relocation, parameters such as walking distance and maximum oxygen consumption improved and persisted for 2 weeks after returning to Jerusalem at 0.91ATA (4). The findings by Kramer et al. were substantiated by 50 years of physiological evidence of the bioactivity induced by pressure changes in the same range as his COPD patients who relocated from Jerusalem to the Dead Sea, moving from 1.0015 ATA to 1.3 ATA (5).

The opposite effect, i.e., worsening of COPD on ascent in "the ocean of air," was observed by Dr. Orval Cunningham, professor of anesthesiology (University of Kansas), the specialty of gas physiology. While on a vacation to the

Colorado Rocky Mountains during the Spanish flu pandemic of 1918, he became aware of the increased mortality of Spanish flu victims at higher altitude (6). He reasoned that, since a descent to lower elevations has a therapeutic effect on patients (2, 3), a further increase in pressure beyond sea level, i.e., to below "the ocean of air," would have further benefit. Using a converted boiler as a hyperbaric chamber in Kansas City, Missouri (altitude 909 ft), Cunningham replicated this improvement caused by altitude descent on human disease when he treated an agonal Spanish flu patient in 1918, achieving complete recovery with four daily 1-h 1.6 ATA hyperbaric air treatment (HBAT) sessions (6, 7) Countless Spanish flu patients followed, and Cunningham extended his clinical treatment benefits to a variety of conditions (7–9). After a hundred years, his reasoning and success were replicated in the application of hyperbaric oxygen therapy (HBOT) to COVID-19 (10–12)· and post-COVID "Long Haulers Syndrome." (13–15).

The results obtained by Kramer et al.· (4) and Cunningham with compressed air (2, 3, 6–9) have been replicated in cerebral palsy children (16, 17), a drowned child (18), and two persistent postconcussion syndrome studies (19, 20). The pressure changes observed in these studies were in the range of the pressure changes observed in the Rocky Mountain/Great Plains relocators (2, 3) and the pressure changes experienced by our dementia patient on weekend trips from his home in the Rocky Mountains to western Nebraska. During these trips, he experienced clinical deteriorations and improvements that tracked altitude changes. Based on the above literature, the authors' personal experience, and the patient's clinical travel experience, the primary author replicated the clinical benefits experienced on the weekend trips to lower altitude (higher pressure) by treating his patient in a portable hyperbaric chamber. In this study, we report the sustained improvement in dementia with repetitive hyperbaric air therapy, using pressure similar to that used by others who have used altitude descent to improve their medical conditions (2–4).

Case description

The patient was a 78-year-old man with dementia who lived for 16 years in Gypsum, Colorado at 6400 feet (1944 m, 0.79 ATA, 11.6 psi) and was a summertime vacation home neighbor of the primary author in Lake McConaughy, Nebraska (3330 feet altitude, 972 m, 0.89 ATA, 13.0 psi). He was cognitively intact until a cerebral injury from an urgent cholecystectomy in the spring of 2019. Post-operatively, the family noticed an immediate cognitive change ("he was no longer himself"). The patient was reduced to social withdrawal, infrequent smiles, and little intelligible speech. He was subsequently diagnosed with dementia. In May 2019, his son-in-law attended a baseball game with the patient in Denver, Colorado (21) (altitude of 5200 feet, 1544 m, 0.83ATA, 12.1 psi) and noticed that he had become incoherent (delirious) around 4:00 PM during the middle innings of the game. Attendance at this game included travel from his home at an elevation of 6400 feet (0.79 ATA) over the Continental Divide (Eisenhower Tunnel, 11,111 feet, 3401 m, 0.66 ATA, 9.6 psi) and a pressure change of−0.13 ATA (see Figure 1). This was the first episode where the patient's sundowning had occurred well before its typical evening time.

Travel to the baseball game entailed 90 min of increasing hypobaric hypoxia from his home at 6400 ft (0.79 ATA) to the Eisenhower Tunnel (11,111 feet, 0.66 ATA) before descent to the baseball stadium at 5,280 ft (0.83 ATA). This included a peak stress of 15–20 min at the Eisenhower Tunnel. The early sundowning was confirmed by additional family members and the primary author's observations on multiple occasions during the summer of 2019 while traveling on the same route to his vacation home in Nebraska for weekend trips (3,330 ft., 0.89 ATA). After staying for a number of days at the decreased altitude (higher pressure), typically on the third day (Sunday afternoons), the family observed behavioral and symptomatic improvement. The primary author also witnessed the patient's cognitive deterioration post cholecystectomy, his further deterioration during visits of summer 2019, and rebounding by Sunday afternoons during vacation home visits.

The observations during the baseball game travel suggested that the hypobaric/hypoxic stress induced by road travel through Eisenhower Tunnel (0.66 ATA) on the patient's dementia (22, 23), a 0.13 ATA decrease in pressure and oxygen pressure, was similar to Cunningham's observations of Spanish flu patients in the Rocky Mountains (6). The lower-altitude higher-pressure rebound improvement observed in the mental status of the patients during Sunday afternoons, a 0.10 ATA increase from his hometown and a 0.23 ATA (3.4 psi) increase from the hypobaric/hypoxic stress zone of Eisenhower Tunnel (0.66 ATA), was in the range of bioactive pressure changes described by Kramer and Godfrey (4) (0.14 ATA increase in pressure), effects of altitude descent from the Rocky Mountains to the Great Plains (2, 3), recompression effects on patients with altitude sickness (24, 25), and the MacDonald–Fraser review of bioactivity in the micropressure range of 1.015–1.3 ATA (5). Based on the authors' previous low-pressure hyperbaric therapy experience with an Alzheimer's patient (26), a drowned child (18), and the medical literature (vide supra), the primary author proposed that the beneficial pressure changes experienced by the patient could be replicated in a portable chamber. Given the effects of HBOT on Nrf2 upregulation (27–29) and of NRF2 effects on intracellular glutathione (GSH) (30), supplemental GSH precursors were recommended adjunctively during hyperbaric treatment.

Treatment

The patient was initiated on daily 1.6 g sublingual glutathione amino acid precursors 3 weeks prior to hyperbaric treatment. The primary author and his wife trained the patient's spouse, children, and adult grandchildren on chamber operations, and hyperbaric treatment was commenced on 20 October 2019, approximately 6 months after cholecystectomy-induced cerebral injury. The treatments were carried out once/daily, 5 days/week, at 1.09 ATA/45–60 min of total treatment time for 20 treatments, 1-week break, repeat 20 treatments. The 0.3 ATA increase in pressure in each hyperbaric treatment was the same increase in pressure used in hyperbaric air treatment of a sub-acutely drowned 2-year-old girl in which global regrowth of brain tissue was demonstrated (18).

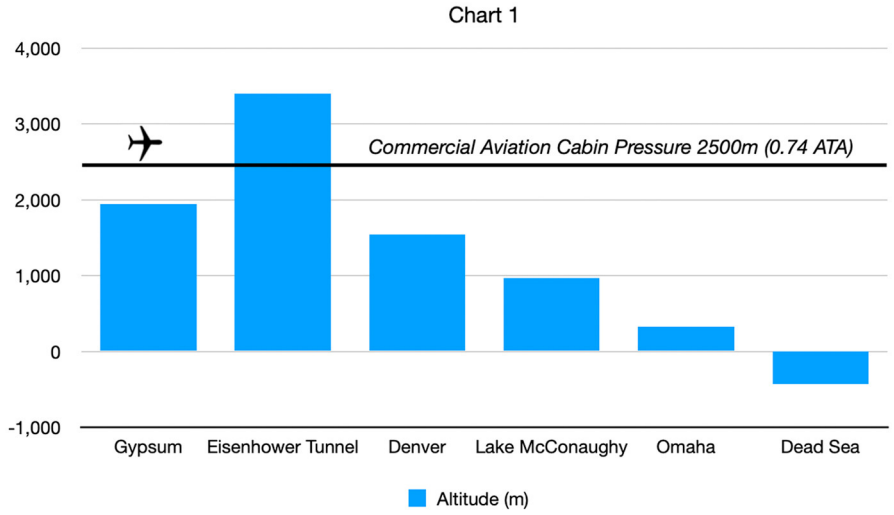

Table 1

Location	Altitude (m)	Altitude (ATA)
Gypsum	1,944	0.79
Eisenhower Tunnel	3,401	0.66
Denver	1,544	0.83
Lake McConaughy	972	0.89
Omaha	323	0.96
Dead Sea	-430	1.05

FIGURE 1
Schematic of relevant altitudes discussed in the case report.

Outcome and follow-up

The patient received 14 hyperbaric treatments in 20 days. After the first four treatments, the improvements in speech, cognition, and activities of daily living were so significant that two television news stations in two different US states reported the patient's progress in separate broadcasts. See broadcast news links shown in Figures 2, 3 and Supplementary material.

The broadcasts demonstrate the return of the patient's quick wit that had disappeared after the cholecystectomy and was still absent on the Sunday before the establishment of hyperbaric/glutathione therapy. The couple humorously referenced the matter of their upcoming 50th anniversary in March of 2020, with the patient stating that it should be remarkably different than what was expected before the establishment of the hyperbaric treatment.

Over the next 4 months, the patient received 67 HBATs at 0–5/week with 11.2 g of GSH precursors. He maintained his cognitive gains and re-engaged in exercising, carried out household chores, and performed activities of daily living until a hospitalization for a urinary tract infection led to him contracting COVID-19 (March, 2020). During the 2-month hospitalization, HBAT and GSH precursors were unavailable, and the patient experienced significant cognitive decline. He was discharged home on multidrug therapy due to worsened dementia/agitation in early May of 2020. He required significant in-home nursing support, was no longer speaking, was frequently combative, and was occasionally delirious, i.e., much worse clinically than when undergoing prehyperbaric therapy and GSH precursors 7 months before. The patient no longer remembered or understood the improvement he had experienced with hyperbaric treatment and refused all attempts at treatment, except for a 1-h session in early June 2020. That single hour of therapy caused an immediate calming of the patient, allowing a trip to the local grocery store later in the day (31). This was his first outing in over 3 months of institutional and home confinement.

Encouraged by the trip to the grocery store, the family traveled to their vacation home (0.89 ATA) the following weekend, incurring another hypobaric/hypoxic stress in the Eisenhower Tunnel (0.66 ATA). Once at the vacation home, the patient went missing, prompting a neighborhood manhunt that found him huddled in the crawl space under his home (31). The family returned to the Colorado home the following morning by the same

FIGURE 2
Colorado/North Dakota television reports links.

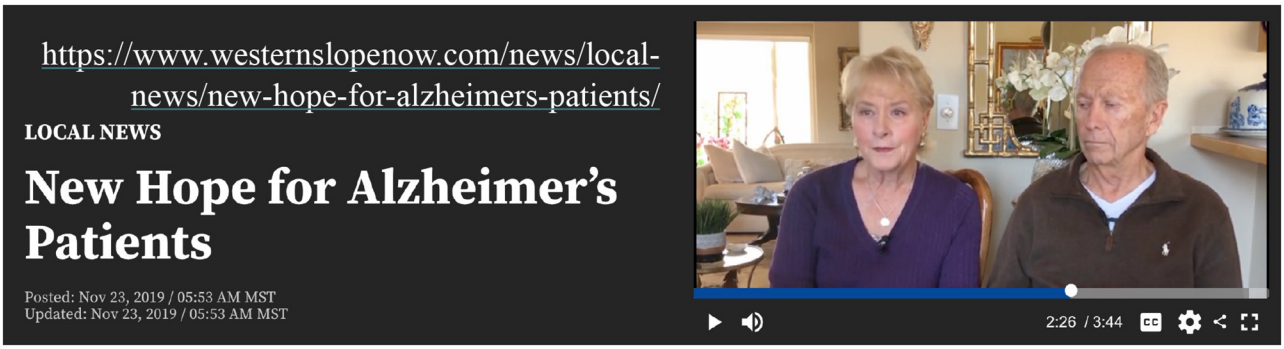

FIGURE 3
11/23/2019 Local media news report of the patient and his wife after 14 hyperbaric treatments. The patient recounts his improvements in activities of daily living. At the 2:26 timestamp of the feature, the patient's spouse relates her husband telling her on day 4 of the therapy, "that machine is working, it's helping me - I know it is." The patient had little to no functional speech before the treatment.

route, incurring another hypobaric/hypoxic stress event through the Eisenhower Tunnel. Upon arrival, agitation worsened over the next several days, necessitating sedation. An extreme nocturnal delirious event occurred requiring physical restraint (88), and the patient expired during sleep within a few days.

Discussion

This case report demonstrates the sensitivity of a patient's dementia to altitude ascents and descents and the replication of beneficial altitude descent conditions with hyperbaric chamber air treatments of the same magnitude. An increase in altitude is a hypobaric/hypoxic stress to living organisms (1, 25). Altitude hypobaric/hypoxic stress is a routine event with nearly every commercial airline flight. Because of in-flight cardiac arrests in patients with coronary artery disease, automatic external defibrillators have been placed in commercial airlines (32). Commercial airline-induced hypobaric/hypoxic stress has also been demonstrated to induce acute delirium in elderly cognitively impaired patients with neurodegenerative disease (33). In younger individuals, Ewing et al. (34) reported cognitive deficits induced by hypobaric/hypoxic stress on asymptomatic college students with a 1–3-year-old mild traumatic brain injury. A traumatic brain injury (TBI) group and a matched control group underwent cognitive testing at 12,467 feet (0.63 ATA) in a hypobaric chamber. Testing at 0.63 ATA demonstrated a statistically significant reduction in cognition in the TBI group compared to the matched controls, and this value was equivalent to acutely concussed patients.

The opposite change in pressure, a decrease in altitude/increase in pressure, is a hyperbaric hyperoxic recompression, similar to the treatment of mountain sickness (25) and divers with

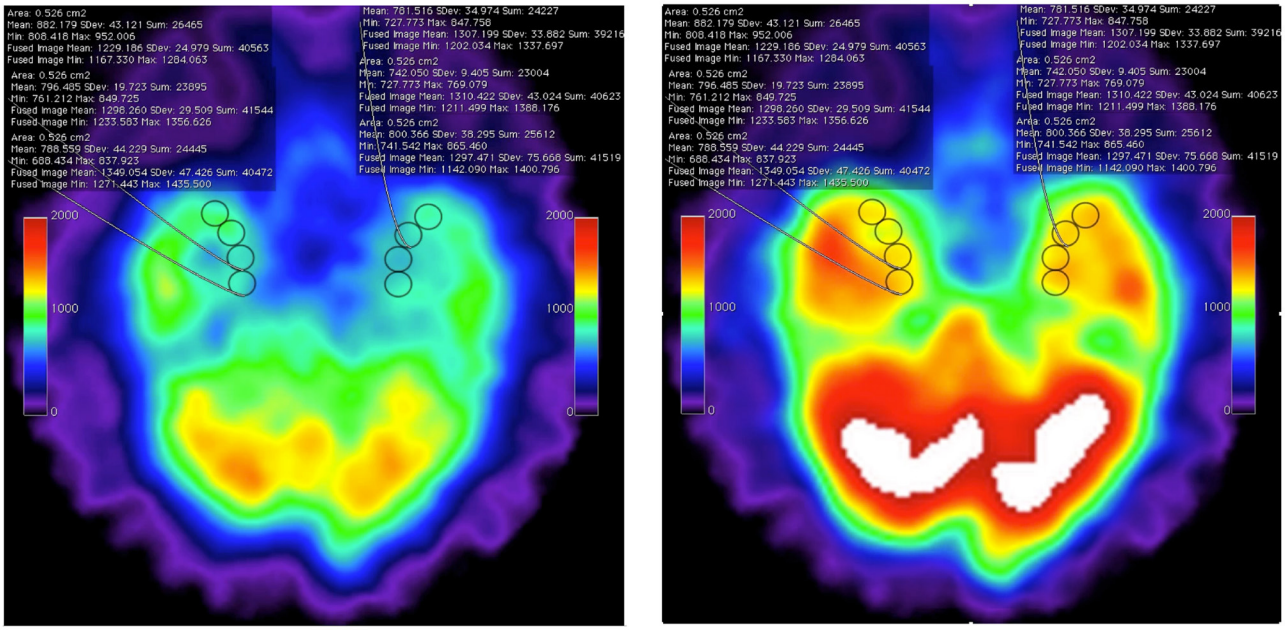

FIGURE 4
Axial SPECT imaging data from pre-HBOT baseline **(left)** and post 1-h HBOT data set **(right)** showing the inferior temporal lobes with eight regions of interest (ROI) in the medial temporal lobes. The statistical overlay documents increased intracellular GSH levels by Tc-99 HMPAO sequestration. These still images are captured from frame 1 and frame 20 of post-processed fusion of the two individual datasets performed on open-source Osirix clinical workstation software (see the source fusion video in Supplementary material). The "fused image" statistical data are obtained from the post-1-h HBOT HMPAO SPECT exam. Note that, at all ROIs, the uptake is increased over baseline by <30% [research data, Harch et al. (59)].

decompression illness (35). Duplicating the experience of demented patients in Sadlon et al.'s review (33), our patient repetitively experienced a deterioration in his dementia symptoms with intermittent trips to over 4,700 feet higher than his home elevation of 6,400 feet (Figure 1). These exacerbations of dementia symptoms were ameliorated by sustained descent to a lower elevation than his home, as reported in the literature (2–4). To improve his dementia symptoms, the same degree of pressure increase in his altitude descents was repetitively duplicated in a home-portable hyperbaric chamber. Each hyperbaric treatment was nearly equivalent to a virtual 1-h transport from his home altitude (0.79 ATA) in the Colorado Rockies to a theoretical landing in the Dead Sea (1.05 ATA), as described for the patients in the study by Krramer et al. (4). Fourteen repetitive hyperbaric air recompressions ("Dead Sea excursions), in combination with glutathione precursors to combat oxidative stress and inflammation, achieved marked cognitive improvement. The improvement was sustained for 4 months by additional hyperbaric and precursor therapy until the patient's demise from COVID-19 brain injury and repetitive hypobaric/hypoxic stress during altitude changes.

This case suggests the bioactivity of the unappreciated component of hyperbaric therapy, barometric pressure, due to the known similar effects in treatment of mountain sickness (25). HBOT has been traditionally defined as the use of 100% oxygen at > 1.4 ATA (35), and more recently > 1.0 ATA (36). These definitions ignored the effect of barometric pressure until the United States FDA pointed this out in 2011 (37). Recent gene expression experiments indicate that barometric pressure may be the dominant component of hyperbaric and hyperbaric oxygen therapy (38). In our patient, the recompression during motor vehicle travel to lower elevations caused equal increases in oxygen and barometric pressure, but the overall pressure of oxygen and amount of oxygen are a fraction of the typical pressures and amounts used in clinical hyperbaric oxygen therapy (36). Such small increases in oxygen and pressure have shown biological (5) and significant clinical effects for altitude sickness (25) but are not appreciated as a treatment for chronic neurological disease. In our case, the initial hyperbaric chamber treatments could not be construed as treating acute injury from the hypobaric/hypoxic trips to over 11,000 feet since our patient had been at his Gypsum home altitude (0.79 ATA) for 6 weeks prior to initiating hyperbaric treatment. The repetitive treatments appeared to duplicate the well-known trophic healing effects of repetitive hyperbaric therapy in other chronic conditions (36). The clinical improvements were durable until acute neurological injury from COVID-19 infection and repetitive hypobaric/hypoxic insults caused his death. His death mirrored the observation of altitude intolerance of chronic disease patients (2, 3, 6) and Dr. Cunnmingham's observation of increased mortality of Rocky Mountain dwelling patients with Spanish Flu (6), a disease very similar to COVID-19.

The precedence for our patient's improvement dates back to the 300-year initial history of hyperbaric therapy, which consisted solely of compressed air usage (6). This experience, long-forgotten by the modern hyperbaric oxygen therapy community, is undergoing a re-validation with accumulating animal and clinical studies documenting the bioactivity of lower pressures of compressed air, oxygen-enriched compressed air, and 100% oxygen

in a variety of conditions (4, 16, 17, 19, 20, 39–43). Our patient's experience and this body of literature strongly indicate HBAT and low-pressure HBOT as therapeutic options for human disease.

The contribution of glutathione (GSH) amino acid precursors to our patient's recovery is impossible to prove but is suggested by the neuroprotective properties of GSH (44). GSH is an essential antioxidant against reactive oxygen and nitrogen species and is critical in regulating mitochondrial function, maintaining cell redox homeostasis, cell cycle regulation, apoptosis, immunological defense, and modulating pathological abnormalities (45, 46). Its deficiency is centrally involved in the pathophysiology of inflammation and many diseases, particularly Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (46–48). The glutathione amino acid formulation administered to our patient has been shown to increase intracellular glutathione (GSH) levels in cell cultures infected with RNA viruses (49).

Intracellular GSH levels are controlled by Nrf2 expression (30). Nrf2 regulates the cell's adaptive response to oxidants and electrophiles (30) and is increased (28, 29) and decreased (50) in diseases and hypobaric/hypoxic stress (51). Nrf2 upregulation has achieved disease amelioration in multiple conditions with multiple therapeutic agents (47, 52). This "systems medicine mechanism-based approach" to disease treatment (53) has described Nrf2 upregulation therapy as a "several diseases, one medicine" therapy; in summary, it is pleiotropic (47). Nrf2 is also upregulated by hyperbaric oxygen therapy in normal human endothelial cells (27) and in diseases (28, 29). We speculate that combining GSH precursors with HBAT caused a synergistic elevation of GSH, improvement of mitochondrial function through a mitohormesis effect, and clinical improvement in our patient. Upregulation of Nrf2 by hyperbaric therapy may explain the potentially unjustified criticism of widespread historical application to 132 diseases (54).

This case report did not include functional imaging documentation. In previous reports, we have documented HBOT-induced changes in chronic neurological disease with functional imaging changes in both SPECT (55–58) and PET (26). For example, the videographic fused images in Supplemental material and bookend still images of Figure 4 show improvement of function greater than 30% across the regions of interest obtained. The fused SPECT data set was obtained as the "prognosticator" data set of the whole brain after the first hour of HBOT at 1.5ATA. These images indirectly suggest the initial overexpression of Nrf2-generated GSH as stimulated by reactive oxygen and reactive nitrogen species (60). In the presented HBAT case, it is surmised that supplemental GSH precursors and hyperbaric air therapy contributed a greater percentage of reactive nitrogen species to the overall effect due to the 4:1 ratio of nitrogen to oxygen in air. SPECT in these cases may have been a proxy for Nrf2 upregulation. The intracellular trapping of the HMPAO SPECT radiopharmaceutical is mediated by glutathione levels in astrocytes (61). The HBOT SPECT brain blood flow and associated clinical improvements may have resulted, in part, from Nrf2-induced increases in astrocyte GSH. In the absence of HBAT and GSH precursors, we further speculate that the patient's cognitive decline post COVID-19 was due to a decline in intracellular glutathione. Glutathione depletion has been suggested as a major cause of the morbidity and mortality of COVID-19 (62).

Conclusion

Cognitive deteriorations and improvements occurred in an elderly dementia patient with hypobaric/hypoxic motor vehicle ascents and descents from altitude. Temporary beneficial motor vehicle descents from altitude were replicated in a portable hyperbaric chamber. Fourteen hyperbaric treatments and oral glutathione precursor supplements resulted in cognitive improvements that were documented in television news mini-documentaries. The improvements were sustained for 4 months with additional hyperbaric and supplement treatment until the patient's hospital admission for UTIs, leading to nosocomial COVID-19 infection. Patient death was due to long COVID-19 dementia exacerbated by repetitive altitude insults. Hyperbaric-induced upregulation of NRF2-mediated glutathione synthesis is suggested as a contributory mechanism of action.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

EF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. PH: Data curation, Methodology, Conceptualization, Formal analysis, Project administration, Investigation, Writing – review & editing.

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Conflict of interest

EF owns multiple hyperbaric chambers and medical imaging devices used in his practice of clinical medicine. From 2016 to 2020, MoPlatte Sports Medicine was the entity under which EF was a distributor for The ProImmune Company, LLC. EF remains a consultant to the inventor of ProImmune Immune Formulation 200, Albert B. Crum, MD (ABC). ABC's Canadian Patent (https://www.ic.gc.ca/opic-cipo/cpd/eng/patent/2963131/summary.html?) was a primary reference for the North Dakota CARES ACT Grant Committee approval of EFF's natropathic/medical countermeasures for COVID19 covered under the United States Federal PREP ACT of 2020. EF is a consultant for Joseph V. Cassarino, the current distributor of ProImmune Immune Formulation 200 at www.theBestImmuneSupport.com

which has provided partial funding support in conjunction with the North Dakota CARES ACT grant support for the broadcasting of the hyperbaric and GSH pre-cursor medical countermeasures to COVID19 developed through EFF's clinical experience in this case on the following FCC regulated communications platforms: KFYR, KXEL, KCRO, WXJB, and KXNET. PH is the owner of an S Corporation that is the vehicle for his practice of hyperbaric medicine. He also performs consulting and gives expert witness testimony under this S Corp.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2024.1356662/full#supplementary-material

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Hypoxic ischemic encephalopathy (HIE)

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Introduction: The morbidity and mortality of acute ischemic hypoxic encephalopathy in newborns have not been dramatically modified over the last 20 years. The purpose of this review is to describe the use of hyperbaric oxygenation therapy (HBOT) in the management of acute ischemic hypoxic encephalopathy in newborns.

Methods: A review of the medical literature was conducted on the use of HBOT in the pathophysiology of this condition and its impact on outcomes of patients treated at an early stage.

Results: When HBOT is administered promptly, it can promote the survival of the penumbra, modulate the cytokine storm, modify inflammatory cascades, restore mitochondrial function, inhibit apoptosis, reinstate cellular communication and cytoskeleton function, reinstall the functioning of the kinase system, reduce cytotoxic and tissue edema, promote microcirculation, and provide an antioxidant effect. All these secondary mechanisms aid in saving, rescuing, and protecting the marginal tissue.

Conclusion: When used promptly, HBOT is a non-invasive adjunct treatment that can preserve the marginal tissue affected by ischemia, hypoxia, meet the metabolic needs of the penumbra, reduce inflammatory cascades, prevent the extension of the damaged tissue, and modulate ischemia-reperfusion injury.

KEYWORDS

acute ischemic hypoxic encephalopathy, hyperbaric oxygenation therapy, mitochondria, ischemia-reperfusion injury, antioxidant effect

Introduction

Hypoxic ischemic encephalopathies (HIE) carry a high risk of death or disability. Annually, 15 million people worldwide suffer a stroke, with approximately 33% of the cases resulting in death and another 33% leading to permanent disability. Despite all the efforts to reduce morbidity and mortality of stroke, the absolute number of strokes continues to increase. One of the most important factors related to the increase in cases appears to be the longer life expectancy observed over the last 20 years. The incidence of stroke increases significantly in patients older than 65 years (1).

The Global Burden of Disease (GBD) study showed that the absolute number of strokes increased by 70% over 30 years (1990–2019). Globally, there was an increase in prevalent cases of 119%, in disability-adjusted life years (DALYs) of 115%, and in annual deaths of 146% (2).

Hypoxic ischemic encephalopathy (HIE) represents a global hypoxic insult to the brain. This complex neurologic dysfunction is estimated to affect 1.5 per 1,000 term births and accounts for 15–35% of all cases of neonatal encephalopathy in preterm and term infants. It includes three phases, namely, the hypoxic/ischemic insult; mitochondrial dysfunction, excitotoxicity, inflammation, and oxidative stress; and cell death, remodeling, repair, and gliosis (3).

Pathophysiology of hypoxic ischemic encephalopathy (HIE)

Life on Earth has evolved in an oxygen-rich atmosphere, and oxygen is essential for breathing, cellular metabolism, cell signaling and communication, inflammation, oxidative damage, and cell death. Many of these processes are mediated by reactive oxygen species (ROS) (4–6).

During hypoxia, oxygen is not available in sufficient amounts at the cellular and tissue levels. The inadequate delivery of blood supply and/or the low oxygen content in the blood will compromise homeostasis. The central core of the lesion will undergo necrosis, while the marginal tissue will suffer from an ischemic and metabolic penumbra. Both penumbras may affect the glial cells and neurons, especially those in the hypothalamus, which are key components in regulating energy homeostasis (7).

Ischemia-reperfusion injury (IRI) is the damage caused by the restoration of blood supply to the tissues (reperfusion/reoxygenation) after a period of ischemia and hypoxia. IRI often results in more tissue damage than the initial ischemic insult. Research has focused on identifying the cellular pathways involved in causing damage to organs by IRI. It is associated with a reduced production of ATP at the ATPase-synthase level, with the concomitant increase in the production of reactive oxygen species (ROS) within the mitochondria (7, 8).

Hypoxia-inducible factor-1α (HIF-1α) is an oxygen-sensitive transcription factor that plays a crucial role in regulating and mediating adaptative metabolic responses to hypoxia and cerebral ischemia. It regulates more than 100 genes and participates in many processes, including metabolism, proliferation, preconditioning, postconditioning, activation of signaling pathways, and angiogenesis. HIF-1α also regulates PI3K, VEGF, EPO, MKP-1, Glut-1, and many glycolytic enzymes, including glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase 1, 12-lipoxygenase, proapoptotic members of the Bcl-2 family (Bnip3), and carbonic anhydrase (9–13).

During hypoxia, mitochondrial ROS production is associated with an increased expression of HIF-1α. The genes that are expressed downstream of HIF activity enhance oxygen-independent ATP generation, cell survival, and angiogenesis. Therefore, they are important factors for tissue protection during IRI. Studies performed in different cell types have shown that HIF-1α regulates hypoxic upregulation of genes, including cyclin D1, transforming growth factor α, POU5F1, and matrix metalloproteinase 2 (MMP-2) (9–13). Therefore, HIFs are rapidly stabilized upon the loss of the oxygen supply, resulting in an orchestrated transcriptional response to modulate cellular phenotypes. This transcriptional response has wide-ranging beneficial effects during the reperfusion of tissues (14).

HIF-1α and HIF-2α share 48% identity. HIF-2α is constitutively expressed in the brain but is not induced by 3 h of hypoxia in the neonatal rat brain. It is expressed mainly in the glial and endothelial cells during normoxia and in less severe hypoxia. The loss of neuronal HIF-2α exacerbated brain injury in the acute (<24 h) and subacute (<6 h) phases of HIE, with a trend toward more severe volume loss in the adult brain. HIF-2α also regulates antioxidant genes, angiogenesis, and cerebral microvasculature reconstruction during brain recovery, playing a beneficial role in maintaining reactive oxygen species and mitochondrial homeostasis (15).

The blood–brain barrier (BBB) is composed of microvascular endothelial cells, astrocytes, neurons, pericytes, and the basement membrane. Oxidative stress during ischemia affects the interaction between endothelium cells and pericytes, leading to blood flow reduction and BBB breakdown. Pericytes appear to be more sensitive to ischemic injury than endothelial cells. HIF-1α inhibition decreases BBB damage by regulating matrix metalloproteinase-2 (MMP-2) and vascular endothelial growth factor (VEGF). The loss of HIF-1 in pericytes reduces ischemia-induced pericyte death, subsequently reducing BBB permeability and CNS transendothelial leakage (16, 17).

HIF-1α could serve a dual role in cell survival or death during cerebral ischemia/hypoxia. It might induce cell death during severe and long-term ischemia/hypoxia but could promote cell survival under mild ischemic stress (9). In the early stages of ischemia, the inhibition of HIF-1α lowers brain injury, edema, and apoptosis. However, in the recovery phase, neuroprotective effects might be achieved by promoting angiogenesis through the induction of VEGF expression (8, 18).

Ischemic reperfusion injury (IRI)

Hypoxic ischemic encephalopathy (HIE) not only generates an ischemic and metabolic penumbra but also creates an ischemia-reperfusion injury (IRI). The impact of ischemia/hypoxia is not uniform across the brain, with several local and systemic factors determining the magnitude of the damage and its potential reversibility. Two key factors involved in the reversibility of the injury are ATP levels and time. Once the mitochondrial oxidative phosphorylation system (OXPHOS) is compromised, a series of detrimental events occur, often simultaneously and/or sequentially (18).

Mitochondria are responsible for ATP production, metabolism, cell signaling, and energetic regulation. An energetic balance is needed to meet the energy needs during normal and stressful conditions. The brain is particularly vulnerable to hypoxia because it consumes 20 times more ATP (4.7×10^9) than the rest of the body (19). Mitochondria also chelates calcium (Ca^{++}) from the cytosol when ion pumps fail. It is also responsible for producing most of the reactive oxygen species (ROS), cell-cell communication, intrinsic apoptosis and maintaining the redox balance. It is also the only organelle that has DNA and shares 30% of its DNA with the nucleus. It is theorized that this is an adaptative cell process to maintain adequate energetic balance, homeostasis,

allostasis, and allostatic responses, which are key for enduring stress conditions (20–22).

One of the mechanisms used to survive low oxygen levels is the severe depression of the metabolic rate during oxygen deprivation in association with lower rates of ATP production via the fermentative pathways (the Warburg effect), which become a key strategy for survival (21). The reduction in ATP availability affects membrane ion pumps, the expression of phosphatases, kinases, transcription factors, and microRNAs (22, 23).

The dysfunction of mitochondrial membrane ion pumps affects cellular ion exchange (Na^+/K^+, Na^+/Ca^{++}). Sodium (Na^+) increases in the cytosol and promotes cytotoxic edema. Brain swelling is also associated with the influx of water into the perivascular astrocytes through channels called aquaporins (AQP). Cerebral ischemia promotes the influx of Na^+ through SUR1-TRPM4-induced Ca^{++} transport into the cells, which increases the calmodulin-dependent translocation of AQP4 to the plasma membrane and water influx into the cell (24, 25).

NA^+ acts as a second messenger that regulates OXPHOS function, redox signaling, and the production of ROS by modulating the fluidity of the inner mitochondrial membrane. A conformational shift in mitochondrial complex I during acute hypoxia drives acidification of the matrix and releases free Ca^{++} from calcium phosphate precipitates. The activation of the mitochondrial Na^+/Ca^{++} exchanger promotes the import of Na^+ into the matrix. This reduces the mobility of free ubiquinone between complexes II and III with the subsequent production of superoxide (O_2^-) in complex III (24).

During hypoxia, a mitochondrial paradox arises. ROS are produced at low oxygen levels. Many observations indicate oxidative stress and/or redox imbalance during low oxygen stress. This is possibly caused by the "electron scape" from the respiratory chain. Complexes I, II, and III are the main mitochondrial sources of O_2^-. It is proposed that several conditions must occur for this paradox. There must be changes in complexes I and III and the participation of NADPH. Moreover, complex II switches its catalytic activity from succinate dehydrogenase to fumarate reductase, creating ROS generation because fumarate reductase is a powerful O_2^- generator (24).

As hypoxia progresses and mitochondria dysfunction increases with a concomitant reduction of ATP beyond a critical level (<1 mol/kg), necrosis occurs. In the hypoxic/anoxic state, cytochrome C is separated from the internal membrane, and the transition pore opens with the subsequent release of Ca^{++} into the cytosol. It is then when the intrinsic apoptotic cascades mediated by Caspase 3 become irreversible, and programmed cell death type I (apoptosis) occurs. Moreover, TNF-α promotes apoptosis via the extrinsic pathway by interacting with FAS receptors and ligands on the surface of cells (22, 26).

Ferroptosis is another programmed cell death caused by an imbalance in iron metabolism and lipid peroxidation by ROS. Due to the Fenton reaction, non-transferrin-bound iron (NTBI) generates free radicals, leading to lipid peroxidation and triggering ferroptosis. It is a key mediator of cortical mitochondrial damage, hippocampal neuronal death, and neonatal HIE. Newborns are susceptible to ferroptosis due to their abnormal iron metabolism, decreased activity of antioxidant enzymes, and the accumulation of ROS (18, 19).

Ca^{++} ions also act as second messengers and are one of the primary mediators of inflammation. Once the cell enters this energetic crisis, calcium influx into neurons stimulates the glutamate receptors, which activate de nitric oxide synthase isoforms (nNOS and iNOS), causing brain injury. Nitric oxide (NO) permeates membranes and reaches mitochondria, which react with superoxide $(O_2)^-$ to yield peroxynitrite ($ONOO^-$). Excess of intracellular calcium also increases mitochondrial superoxide and hydrogen peroxide formation. High mitochondrial oxidant levels can overwhelm the mitochondrial antioxidant system, promoting the generation of stronger oxidants, such as hydroxyl radicals ($^{\cdot}OH$). NO is also involved in the regulation of hypoxia-related genes and might stabilize hypoxia-inducible factor (HIF), a key component of hypoxic acclimation (22, 23, 26–29).

Calcium also mediates other inflammatory cascades. It activates calcium-dependent proteases, which mediate the conversion of xanthine dehydrogenase to xanthine oxidase, enhancing the production of ROS. Calcium also stimulates phospholipase-2 with the subsequent elevation of cyclooxygenase (COX), lipoxygenase, leukotriene, thromboxane, and prostaglandins. Another cascade is the cytokine cascade, mediated by nuclear transition factor kappa B (NFkB). It mediates approximately 150 different cytokines, both inflammatory and anti-inflammatory. It is not well understood how NFkB promotes inflammatory or anti-inflammatory cytokines, but it might involve modulating the redox balance of the cell. It also promotes the production of endothelins, chemokines, interferon, transcription factors, metalloproteases, heat shock proteins, glutamate, caspases, HIF-1α, and NO (22).

The endothelium and the extracellular matrix (ECM) integrate many functions and are probably the first alarm system of the body. Ischemia, hypoxia, and hypoglycemia stimulate the expression of intracellular adhesion molecules (selectins, VCAM, and ICAM-1), the neutrophil integrin-β2, and the release of signaling molecules (cytokines, endothelins, chemokines, transcription factors, kinases, and growth factors) (11). An important reduction in the bioavailability of NO is one of the most important factors in endothelial dysfunction. Oxidative stress leads to eNOS uncoupling and promotes the production of superoxide instead of NO (30).

Endothelial cells possess mechanical, shear stress, and biochemical sensors. The structural and biochemical changes in the endothelial cells contribute to neuroinflammation (31). The blood flow is reduced during ischemia, which, in turn, reduces shear stress, stimulating changes in the actin network of the endothelial cells and resulting in the formation of stress fibers (32). Ischemia also induces changes in F- and G-actin, leading to the reorganization of the cytoskeleton (33). The changes in the cytoskeleton also affect the cell-cell junctions, especially the tight and gap junctions, which are significantly affected by hypoxia. The blood–brain barrier (BBB), an endothelial system, is particularly prone to dysfunction during hypoxia.

There is also a crosstalk between cellular geometry and TNF-α signaling. TNF-α induces geometry-dependent actin depolymerization, enhancing IkB degradation, NFkB translocation, and a geometry-dependent-gene expression pattern (34). These observations confirm the participation of tensegrity during

ischemia and hypoxia, where form creates a function and function generates form.

The plasma membrane allows the cell to sense and adapt to changes in the extracellular environment. One of the critical cellular signaling pathways involved in this adaption is the Hippo pathway. This pathway acts as a nexus and integrator of cellular responses to tension, stretching, and changes in the extracellular matrix (ECM) properties. The activation of the Hippo pathway and transcriptional changes through the MST/YAP/FoxO pathways can lead to apoptosis (35). Several components of the Hippo pathway, including YAP, TAZ, KIBRA, LATS1/2, and MST1/2, can temporarily localize to junctional complexes, establishing a good interplay between cellular junctions and the Hippo pathway (36). The sensors, effectors, and transcription factors within this pathway respond to various stimuli and adjust to new and critical environmental conditions. During ischemia and hypoxia, numerous stimuli are produced at the plasma membrane. There must be a system that coordinates, synchronizes, and prioritizes these signals. This coordinating system appears to be the kinase system, which includes phosphatases. Two of the most important kinases in hypoxia are AMP-activated protein kinase (AMPK) and serine/threonine kinase (AKT) (37).

Hypoxia is accompanied by nutrient starvation. Hypoxic signaling is closely linked to nutrient signaling. In nutrient signaling, AMPK and the mechanistic target of rapamycin complex-1 (mTORC1) crosstalk to sense cellular ATP, glucose, and amino acid levels. AMP is a more sensitive indicator of cellular energy states than ATP and activates AMPK. AMPK acts by activating catabolic pathways to facilitate ATP generation once its cellular levels start to decrease. AMPK and mTORC1 modulate several cellular responses during stressful conditions, including mitochondrial respiration, ROS production, protein translation, metabolic reprogramming, and programmed cellular death Type II (autophagy) (37, 38).

AKT stimulation leads to temporal phosphorylation profiles in endothelial cells, affecting growth factor signaling, angiogenesis, cellular protection against oxidative stress, and neuronal damage. Growth factors and insulin stimulation lead to the activation of phosphoinositide-3-kinase (PI3K), the principal mediator of autophagia. The recruitment of AKT to the plasma membrane promotes the phosphorylation of several substrates and residues of Thr308 and Ser473 of rapamycin complex 2 (mTORC2), which are essential for AKT activity. AKT signaling plays a significant role in cellular protection against oxidative stress and neuronal damage (39).

Mitochondria can also actively regulate innate immune responses in infections and sterile conditions. It can directly activate the immune response and modulate it. Pathogen-associated molecule patterns (PAMPs) serve to identify organisms as foreign. Damage-associated molecular patterns (DAMPs) are released or modified during mitochondrial damage in hypoxia. They are recognized as alarmins by receptors of the innate immune system and trigger the immune response. It appears that the loss of mitochondrial membrane integrity, resulting in the escape of components into the cytosol, is the driving force behind this response. However, the exact mechanism is not yet well understood. ATP is included in the mitochondrial alarmins when it is expelled extracellularly by apoptotic or necrotic cells and sensed by the P2X7 receptor to trigger innate immune responses, including the NLRP3 inflammasome (39).

The families of TLR are transmembrane proteins that recognize endogenous ligands and participate in inflammation pathways. Mitochondria are implicated in TLR signaling through TNF receptor-associated factor 6 (TRAF6). Mitochondrial gene expression is upregulated downstream of both TLR3 and TLR4 through the activity of the PPAR-y coactivator family. TLR-4 activation during stress-induced hypoxia of neuronal cells induces an inflammatory response through the formation and activation of autophagy and NLRP3 inflammasomes, which induce IL-1ß release. TLR4 knockdown significantly suppresses the expression of TLR and inhibits apoptosis (39, 40).

Besides cytokines, signaling molecules, leukocyte migration, activation, and the complement cascade compose the innate immune system. In HIE, cytokines are central to the propagation of the immune response, particularly IL-1ß. This can result in direct neural injury that culminates in cell death (pyroptosis). Leukocyte chemotaxis and activation are central to the second phase of the cerebral ischemic reperfusion injury (IRI). The activation of the complement cascade facilitates the activation of leukocyte and endothelial cells and an increase in the release of cytokine. Complement participation after cerebral IRI plays a role in the classical, alternative, and lectin pathways (41).

Animal models of hypoxic ischemic encephalopathy (HIE) and hyperbaric oxygen therapy (HBOT)

Most of the experimental data come from the neonatal hypoxia-ischemia model developed by Rice and Vannucci in rats (42). The animal data show promising applications of HBOT in acute HIE. In Table 1, we summarize the results of animal HIE and HBOT (43–61).

Hyperbaric oxygen therapy (HBOT)

HBOT is a treatment where a patient breathes 100% oxygen inside a pressure vessel designed for human occupancy, either a monoplace or multiplace hyperbaric chamber, at ambient pressures ranging from 1.5 to 3.0 atmosphere absolute (ATA). Each treatment session takes 60–120 min and can be conducted one to three times daily, depending on the medical condition of the patient. Acute pathologies, such as CO poisoning, might require one treatment, and chronic osteomyelitis might take 40 treatments. Only one treatment might be needed in the early management of acute HIE (within 4–6 h after delivery). The subacute cases might require more.

HBOT is based on gas laws, particularly Henry's law. The primary mechanism of HBOT is hyperoxygenation. The plasma partial pressure obtained at 2.0 ATA is close to 1,500 mmHg and close to 2,000 at 3 ATA. This hyperoxygenation effect creates temporary oxidative stress during the first 60 min of treatment, but it also creates an important antioxidant effect that remains for 72 h after the last treatment (62–64).

TABLE 1 Use of HBOT in animal models of HIE.

References	Model	N	Area/Mediator	Treatment protocol	Results (SA)	Comments
Wei et al. (43)	7-day-old rat pups in 3 groups (sham, HI control, and HI_HBO). Carotid ligature, 2h 8%O_2	120	Pyramidal cells, glial cells, and hippocampal dentate gyrus	HBO/min/QA	$p < 0.001$, $p < 0.05$	Promoted repair and regeneration of the nervous system and contributed to the self-recovery and protection of damaged brains
Xue et al. (44)	Adult rats. Sham, control, HI, Hi-1.5ATA, and HI-2.5ATA	60	Hippocampus. Prefrontal cortex. IL1ß, IL6, TNFa, HIF1a, and SOD	HBO 1.5–2.5 ATA/60 min/QA for 6 days	$p < 0.01$, $p < 0.05$	Protects myelin injury, promotes differentiation into oligodendrocytes, inhibits neuroinflammation, and balances oxidative damage and antioxidant activity; 2.5 AT A showed better results than 1.5 ATA
Wang et al. (45)	7-day rat pups, carotid ligation/cut + 2 h hypoxia, study 30 days	70	HBO at 3,6,12,24, and 72 h. Stem cell proliferation and behavior evaluation	HBO 2.0 ATA/60 min/QD for 7 days.	$p < 0.05$	Increases proliferation of neural stem cells, performs better in behavior tests, and causes less neural loss in hippocampal CA1; best at <12 h.
Chen et al. (46)	7-day rat pups, carotid ligation/cut + 2 h hypoxia, HBO at 1 h	80	Brain histopathology 7 days, water maze test 30 days; caspase 3 Nogo-A, +water maze test	HBO 2.5 ATA/120 min/QD for 7 days; w/ephedrine	$p < 0.01$, $p < 0.05$	Reduces Caspase 3, Nogo-A and improves the Morris water maze tests after 4 weeks.
Chen et al. (47)	14-day-old rats, SOD, MDA, VEP	40	Antioxidant, lipid peroxidation, brain synapsis CA3, and P1 VEP	HBO 2.0 ATA/60 min/QD for 14 days.	$p < 0.01$, $p < 0.05$	Enhances antioxidant capacity, less ultrastructural damage, improves synaptic reconstruction, and promotes brain function
Yin et al. (48)	7-day rat pups, BMP-4, HBO at 6 h HIE	30	BMP-4, nestin, tunnel in the hippocampus	HBO 2.0 ATA/40 min/QD for 7 days	$p < 0.01$	Promotes neurological recovery and inhibits neural apoptosis.
Feng et al. (49)	7-day rat pups, BMP-4, nestin, tunnel in the hippocampus HBO at 6 h HIE	108	Brains removed at 1,3,5,7,14,21 days	HBO 3 ATA 7 60 min/QD for 7 days	$p < 0.05$, $p < 0.01$	Proliferates neural stem cells, improves recovery, and increases nestin
Wang et al. (50)	7-day rat pups, Wnt 3, nestin, subventricular zone, HBO at 6 h HIE	150	Stem cells, nestin, WNR-3, at 6,24, and 7h and 7,14 days	at 3 h HIE HBO2 ATA/60 min/QD/	$p < 0.05$, $p < 0.01$	Proliferation/migration of neural stem cells subventricular zone, increased nestin, and Wnt-3
Wei et al. (51)	7-day rat pups, NGF, HBO at 6 h HIE	40	NGF, water maze, sensory-motor function at 30 and 42 days	HBO 2.0 ATA/30 min/QD for 7 days	$p < 0.01$	Improves synaptogenesis, dendritic changes, and synaptic remodeling. Reduces escape latency and piercing index and improves sensory motor function
Yang et al. (52)	7-day rat pups, NGF, HBO at <1 h HIE	360	Mitochondrial function/MTP at 0,2,4,6, and12 h 2,3,4,5,6, and 7 days	HBO ATA/1 h/QD for 7days	$p < 0.05$	Mitochondrial function/MTP after HBOT single dose at 2,3,4,5,6 and 7 days
Li et al. (53)	7-day rat pups, HBO at 24 h before HIE		Caspase 3,9, TTC, and tunel	7-day rat pups, HBO, and HBO 2.5 ATA/150 min	$p < 0.05$	Preconditioning HIE increases survival and reduces infarction apoptosis
Chen et al. (54)	7-day rat pups, HBO < 24 h	21	Wnt3 -ß-catenin, BMP. NSC to neuron, oligodendro-cytes	HBO 2.0 ATA/60 min	$p < 0.05$	Promotes differentiation of NSC into neurons, oligodendrocytes, increases astrocytes through Wnt-3/ß-catenin, BMP2. HBO 1 h > 30 min, 2 ATA > 3 ATA

(Continued)

TABLE 1 (Continued)

References	Model	N	Area/Mediator	Treatment protocol	Results (SA)	Comments
Calvert et al. (55)	7-day rat pups, HBO at 1 h	30	Caspase 3 at 18–24 h, PARP at 18–48, Tunel, cortex, and hippocampus, brain removed 12,18,24, and 48 h at 4–6 weeks.	HBO 3 ATA/60 min	$p < 0.05$	Increases neuroprotection, reduces apoptosis and Caspase 3 cortex, hippocampus, reduces DNA fragmentation, tunel, and preserves brain weight,
Calvert et al. (56)	7-day rat pups, HBO at 1 h	144	ATP, ATPase, hippocampus, and cortex at 4,24, and 72 h	HBO 2.5 ATA/120 min	$p < 0.05$	Reduces IRI, brain weight, and morphology, 2.5 ATA > NBO
Calvert et al. (57)	7-day rat pups, HBO at1 h	144	ATP, ATPase, hippocampus + cortex at 4,24, and 72 h	HBO 2.5 ATA/120 min	$p < 0.05$	Reduces IRI, brain-weight and morphology, 2.5 ATA > NBOT
Calvert et al. (58)	7-day rat pups	134	at 1,1.5,3 ATA histology at24h, 1,2, and10 weeks and 2, HIF1∞, and VEGF	HBO1.5-3 ATA/60 min	$p < 0.05$	Protects against retinopathy, retinal vascular density, < brain weight, no > HIF1∞, VEGF
Günther et al. (59)	Hypoxically damage rat neocortical brain slices at 5-30 min hypoxia	-	HPLC, Celestine blue/fuchsin staining, ATP/ADP, GTP/GDP	HBO 2.5 ATA/30–120 min	$p < 0.05$, $p < 0.01$	HBO 2.5 ATA/60 min > NBO at 5 min better than 30 min
Liu et al. (60)	7-day rat pups, HBO at 1 h	108	Neuroprotection at 28–60 days, histology, Caspase3, AIFat 12,24, and 48 h, sensorimotor, Morris water maze test, and tunel	HBO2.5 ATA/90 min/QD	$p < 0.05$, $p < 0.001$	HBO promotes long-term functional and histological recovery, induces neuroprotection, suppresses apoptosis, and inhibits Caspase-3 and AIF pathways
Calvert et al. (61)	7-day rat pups, HBO at 1 h	151	Ipsilateral hemisphere weight, sensorimotor tests at 5 weeks	HBO 3 ATA/60 min	$p < 0.05$	HBO reduces atrophy, and apoptosis and improves sensorimotor function at 5 weeks

Hyperoxygenation produces several secondary mechanisms. Hyperoxygenation increases the diffusion of oxygen from the vascular space into the tissular space, restoring ATP production and cellular and tissular oxygen tension and promoting cell survival in the ischemic and metabolic penumbra (62–64).

It also breaks the vicious cycle of edema-hypoxia-edema. The edema-reduction effect is also caused by arterial vasoconstriction. It depends on the partial pressure of oxygen and the tissue involved. Vasoconstriction is more pronounced in the central nervous system (65). HBOT enhances K-ATPase activity, increases active NA^+ transport, and accelerates edema clearance. It also reduces endothelial damage and restores cell-to-cell junctions (22, 24). HBOT reduces the mRNA and protein expression of aquaporin (66). The reduction of tissue edema improves cerebral microcirculation.

HBOT facilitates the correction of mitochondrial dysfunction, improves the integrity of compromised mitochondrial membranes, and inhibits secondary cell death by causing the transfer of mitochondria from astrocytes to neurons. It also upregulates ATP expression through increased NAD^+ expression, an important marker of energy metabolism. There is an upregulation of the Sirt1 expression with a concomitant reduction of the expression of p53 and NFkB. The restoration of the mitochondrial transmembrane potential by HBOT is associated with a significant reduction of the intrinsic apoptotic cascade mediated by caspase 3 and caspase 9 and an increased expression of Bcl-2 and Bcl-xL that inhibit apoptosis. There were no significant changes in the levels of the proapoptotic protein Bax, as Bcl-2 inhibits it (66–68).

HBOT has also been shown to reduce calcium overload, which is associated with a reduction in the intracellular calcium level and the inhibition of autophagy through decreased expression of p53 mRNA, AMPK, and mTOR (40). HBOT attenuates the increase in IRI and HIE of IL-1ß, IL-6, IL-8, INF-Y, TNF-α, HIF-1α, ICAM-1, and Integrin-ß2 ($p < 0.05$) (65, 69, 70). It has also shown protective effects by increasing the levels of eNOS. HBOT attenuates neuroinflammation not only by reducing the secretion of proinflammatory cytokines but also by increasing the expression levels of the anti-inflammatory cytokines IL-4 and IL-10 (66–70). HBOT modulates neuroinflammation by decreasing the expression of CCL2/CCR2, matrix metalloproteinase-9, and TNF-α. It also inhibits secondary brain injury by activating the TLR4/NFkB, JNK, p38-MAPK-CCR2, and ERK signaling pathways (45). The effects of HBOT on HIE and IRI are shown in Figure 1 (22, 26).

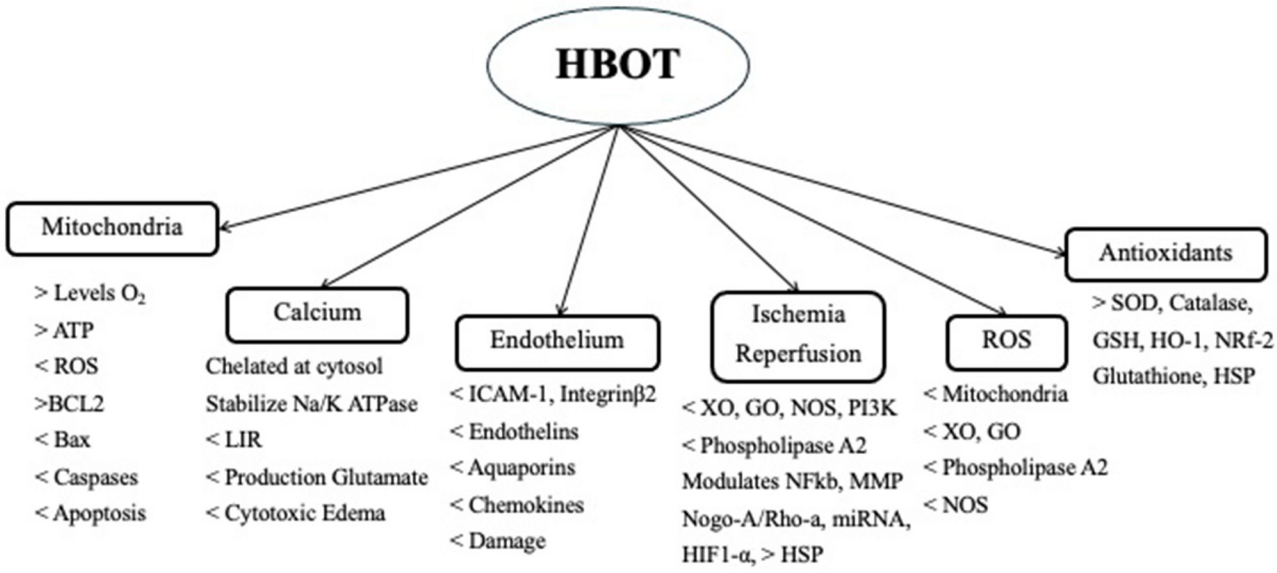

FIGURE 1
Use of HBOT in IRI.

HBOT promotes a variety of antioxidant enzymes. It induces the activation of transcription factors and the gene expression of antioxidants. It induces Nrf2, which is a redox-sensitive transcription factor that acts on genes of Hem-oxygenase-1 (HO-1), quinone oxidoreductase 1, and glutathione S-transferase, reducing the ROS load. HO-1 is also known as HSP-32 and has a neuroprotective effect. HBOT protection is also enhanced by increasing the levels of glutathione, superoxide dismutase (SOD), catalase glutathione peroxidase (GPx), and reductase (GR) (68, 71–73). Both GPx and GR are significantly increased with HBOT and are negatively correlated with infarct volume ($p < 0.01$ and $p < 0.05$, respectively) (Figure 1) (73, 74).

HBOT has also shown beneficial effects on acute (75–77), subacute (78), and chronic stroke (79). There is still no good evidence to suggest that HBOT should be the gold standard for managing acute stroke, but its clinical benefits cannot be entirely ruled out. New research studies show that HBOT reduces and improves functional symptoms, improves mobility, and reduces treatment time for patients (80).

HBOT in neonatal HIE and IRI

The first cases of hypoxic ischemic encephalopathy (HIE) treated with hyperbaric oxygen therapy (HBOT) were reported in the first half of the 1960s (81, 82). This practice continued in the former USSR and was reported in the early 1980s, with more than 1,400 cases treated (83–85). The use of HBOT in neonatal HIE was discontinued for a long period due to technical factors, logistics between the neonatal and HBO departments, little experience in pediatric and neonatal patients, and fear of side effects.

Further publications were reported in the early 2000s from Mexico and China (86–88). The Mexican group recommended not treating patients under 34.5 weeks of pregnancy and <1.2 kg of weight due to the higher lung and eye complications related to prematurity. They also published the proposed HBOT protocol for neonatal HIE (86). Neonatal HIE is more frequently encountered in neonates with an Apgar score under 3 at 1 min and 5 at 5 min, with a pH lower than 7.2, and a resuscitation time longer than 8 min. Neonates presenting with these conditions will develop cerebral edema at 4 h and convulsions at 6 h post-delivery. Due to the severity of this condition, neonates require NICU care that must be continued during HBOT (86).

Similar to other ischemic/hypoxic conditions, the advantages of HBOT in HIE in neonates are time dependent. HBOT should be initiated as early as possible, preferably within the first 4 h, but ideally during the 1 h after delivery. Furthermore, the treatment pressure should be between 1.5 and 1.8 ATA with a duration of 45 to 60 min to reduce possible HBOT side effects. Only one treatment is needed when HBOT is applied within the first 4 h after delivery (86).

A systematic review conducted in China reported that HBOT reduced mortality and neurologic sequelae in term neonates with HIE. Their protocol involved using HBOT at 1.5–1.7ATA for 60 to 90 min, one to three times a day. HBOT was administered within 24 h in most cases, but the exact time to administer it was not specified. The results suggested that HBOT may reduce mortality (OR 0.26, CI 95% 0.14 to 0.46) and neurologic sequelae (OR 0.41, IC 95% 0.27 to 0.61). According to the authors, the reports were of poor quality and suggested the need for adequately powered, high-quality, randomized controlled trials (88).

In another article published in Chinese, they presented their experience with 60 patients treated with three different treatment pressures (1.4, 1.5, and 1.6 ATA) for 60 min, once a day for 7 days. They measured serum levels of malondialdehyde (MDA), SOD, NO, and NOS before and after HBOT. Serum SOD levels increased, while serum levels of MDA, NO, and NOS decreased ($p < 0.05$). The neonatal behavioral and neurological assessment

(NBNA) scores in the three groups increased significantly after HBOT ($p < 0.05$). There were no side effects reported (89).

A meta-analysis was conducted in China with 46 clinical RCTs that included 4,199 patients with neonatal HIE treated with HBOT. Their results indicated that HBOT significantly improved the total efficiency (TEF) of treatment in neonates with HIE (OR 4.61, 95%CI 3.70 to 5.75 – $p < 0.0001$), reduced the risk of sequelae (OR −0.3, 95%CI 0.16 to 0.33, $p < 0.0001$), and increased the NBNA scores (MD −4.51, 95%CI 3.83 to 5.19, $p < 0.0001$). They concluded that HBOT is a potential complementary treatment for neonatal HIE, but the study protocols had great heterogeneity (89). There was no information on the time to start HBOT after delivery, and the range of treatments was 5 to 90, at pressures ranging from 1.4 to 1.6 ATA once a day (88).

In another Chinese article involving 80 patients with neonatal HIE, HBOT was associated with monosialotetrahexosylganglioside sodium (GM1), a neurotrophic factor extracted from the porcine brain. The patient was > 2.5 kg, and gestational age ranged from 37 to 41 weeks. All the patients were treated within 12 h of birth. The treatment pressure was kept between 1.3 and 1.5 ATA with 80% oxygen for 20 to 25 min, once a day in a 10-day cycle, 1 week apart, and three cycles in total. They concluded that GM1 combined with HBOT can significantly improve both short-term and long-term nervous system development and brain physiology in children with moderate and severe HIE (90). Despite their good results, the oxygen treatment does not fulfill the definition of hyperbaric oxygen due to the treatment pressure (1.3 ATA), treatment time of 20–25 min, and the use of oxygen at 80%.

HBOT side effects in neonatal HIE

In general, term neonates have good antioxidant defenses. Neonates have the highest antioxidant defenses encountered in life in preparation for breathing oxygen at birth. Nevertheless, it is not the same for premature babies, especially those under 34.5 weeks of gestation (86). It has been reported that HBOT might prevent the retinopathy or retrolental fibroplasia of premature babies (91–93). In the articles reviewed, there were few side effects of HBOT in neonatal IHE. It is probably related to the reduction of IRI and the low treatment pressure used. In the event of pulmonary oxygen toxicity, pulmonary surfactant should be readily available to adequately and promptly manage it. A bi-spectral index monitor (BIS) could be used to monitor the EEG during the treatment to monitor probable central nervous system (CNS) poisoning. Retinopathy does not appear to be a problem unless the neonate is under 34.5 weeks of gestation.

Other treatments for neonatal HIE

A systematic meta-analysis of 11 randomized controlled studies (RCTs) published in 2013 investigated the effects of selective head cooling and whole-body cooling initiated within 6 h of birth in infants with a gestational age of > 35 weeks and moderate to severe HIE. The analysis found that hypothermia was associated with a reduced risk of death or major neurodevelopmental disability by 18 months of age (RR −0.75, 95%CI 0.68 to 0.83). Long-term follow-up of these studies is still pending (94, 95). Current protocols for hypothermia are only partially effective, with improved outcomes if started within the first 6 h of birth. Despite the beneficial effects of hypothermia, 48% experience devastating complications. Various pharmacological treatments (erythropoietin, allopurinol, melatonin, cannabinol, and exedin-4/exenatide) have been examined for use in combination with hypothermia. However, there is a need for more studies to determine their efficacy (96). Recently, a Chinese article proposed the combination of HBOT and a mild hypothermic mattress, suggesting potential synergistic benefits (97).

Conclusion

Almost all life on Earth depends on oxygen and has to adapt to an oxygen-rich environment. The appearance of antioxidants, chloroplasts, and mitochondria enabled life on Earth. It flourished into the five different lines that inhabit it now. Oxygen has become one of the fundamental components of cell-to-cell communication and signaling through its reactive oxygen species. Many of the cellular responses during normal situations, but especially during hypoxia and reperfusion injury, depend on the cellular redox balance. It governs most of the acute responses during cellular stress and participates in the maintenance of homeostasis and allostasis. Hyperbaric oxygen therapy helps restore oxygen partial pressure at the cellular and tissue levels, supports cell communication and signaling, and maintains the redox balance, especially in critical situations such as hypoxic ischemic encephalopathies and ischemic reperfusion injury.

HBOT has not been systematically used for neonatal HIE due to concerns about treating pediatric and neonatal patients. The possible side effects of HBOT in neonates have hindered its use. The treatment should be applied ideally within the first hour after birth and requires close coordination between the neonatal and hyperbaric medicine departments. It is recommended that a neonatologist be on the hyperbaric team to maintain the same quality of care in the hyperbaric unit as in the neonatal ICU. The hyperbaric team must be trained in the management of neonates, and the neonatal team must know the particularities of HBOT in neonates.

However, there are technical and equipment issues. There are no neonatal IV pumps or ventilators. Thus, to provide IV fluids, we need to turn the pump on and off to guarantee the appropriate volume that neonates need. Since there are no hyperbaric neonatal ventilators, a neonatologist must ventilate the patient with an Ambu bag inside the chamber. To avoid neonatal hypothermia during HBOT, the bed linen should be preheated at 40°C in a vapor autoclave.

Selective head or total body cooling is a standard treatment but must be optimized. It is part of the pathophysiology of HIE, but not all of it. HBOT has a greater impact on hypoxia and ischemia-reperfusion injury than hypothermia in neonatal HIE. It is true that not all hospitals have a hyperbaric chamber, but even in those with

hyperbaric departments that treat acute IRI, patients do not get referred for treatment.

There is a need to expedite treatments to meet the window of opportunity of <6 h, ideally within the first hour after delivery. It could be accomplished if the OBGYN, Neonatal, and Hyperbaric Departments worked together as a real multidisciplinary team. There are many challenges, but it has been proven that a neonate can be treated in the hyperbaric chamber within 30 min of delivery (86).

Since selective head and total body cooling could be improved, it would be favorable to combine both treatments. First, physicians should start with HBOT very early (<1–4 h). When administered during this time window, the Mexican experience showed that only one treatment was needed to reverse HIE. Then, hypothermia could be used to complement and continue the treatment. The patients should be evaluated daily to establish the duration of the hypothermia treatment. Two very strong paradigms can make this happen. First, physicians should change their mindset regarding the acute application of HBOT in HIE and IRI. HBOT is used for several acute ischemic conditions but has not been extended to neonatal HIE. Second, the mindset of neonatologists should be changed to apply HBOT very early and then continue with hypothermia.

Neonatal HIE is a devastating injury that has one of the largest health inequities and carries a large global burden of disease (GBD), especially in moderate and severe cases. Despite efforts, the real morbidity, mortality, and lethality of neonatal HIE have not shown substantial reductions over the past 20 years. Thus, it would be interesting to incorporate other treatments to improve the outcomes of neonatal HIE, not only pharmacological but also HBOT.

Currently, neonatal HIE is not an accepted condition by the Undersea and Hyperbaric Medical Society (UHMS), although other acute ischemic conditions are. This lack of recognition means that medical insurance companies do not reimburse for neonatal HIE, further hindering its use. Finally, the basic science supports the potential benefits of HBOT in treating neonatal HIE, but it is important to develop more clinical trials (RCT) to show its real value and take it from the lab to clinical practice.

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ES-R: Conceptualization, Formal analysis, Investigation, Supervision, Visualization, Writing – original draft, Writing – review & editing. VL: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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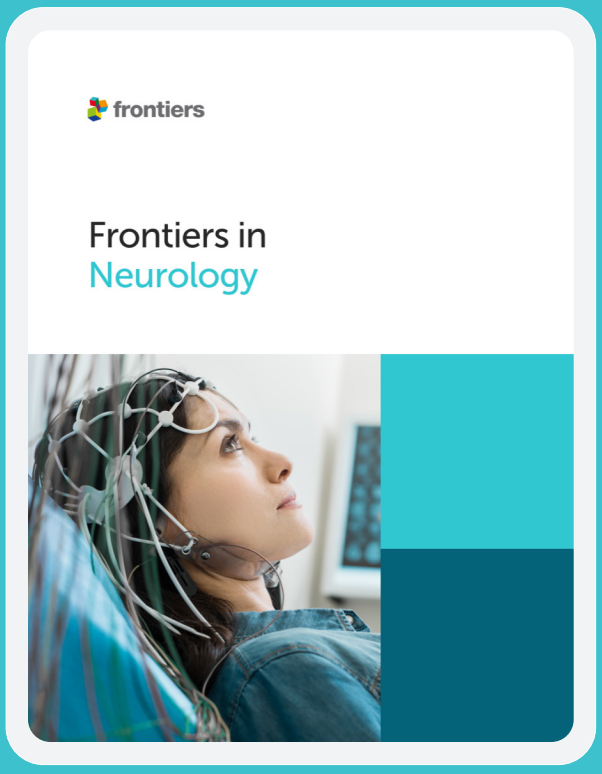

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