Mechanisms of hyperbaric oxygen and neuroprotection in stroke

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Abstract

Cerebral vascular diseases, such as neonatal encephalopathy and focal or global cerebral ischemia, all result in reduction of blood flow to the affected regions, and cause hypoxia–ischemia, disorder of energy metabolism, activation of pathogenic cascades, and eventual cell death. Due to a narrow therapeutic window for neuroprotection, few effective therapies are available, and prognosis for patients with these neurological injuries remains poor. Hyperbaric oxygen (HBO) has been used as a primary or adjunctive therapy over the last 50 years with controversial results, both in experimental and clinical studies. In addition, the mechanisms of HBO on neuroprotection remain elusive. Early applications of HBO within a therapeutic window of 3–6 h or delayed but repeated administration of HBO can either salvage injured neuronal tissues or promote neurobehavioral functional recovery. This review explores the discrepancies between experimental and clinical observations of HBO, focusing on its therapeutic window in brain injuries, and discusses the potential mechanisms of HBO neuroprotection.

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1. Introduction

Hyperbaric oxygen (HBO) has been used in multiple neurological diseases, including cerebral air embolism, carbon monoxide poisoning, vegetative state, global cerebral ischemia caused by cardiac arrest, focal cerebral ischemia, acute spinal cord injury, chronic brain injury, and cerebral vasospasm after subarachnoid hemorrhage. The safety of HBO has been tested in all age and gender groups, including neonates and pregnant patients. The mechanisms of HBO on neuroprotection include improvement of brain metabolism, reduction of blood–brain barrier permeability and brain edema, decreasing intracranial pressure, attenuation of inflammatory response, and prevention of apoptotic cell death. In addition, HBO produces ischemic tolerance or preconditioning neuroprotection.

In this review, we will focus on the application of HBO in neonatal hypoxia–ischemia, focal cerebral ischemia, and global cerebral ischemia. Additionally, we will discuss experimental data on the mechanisms of HBO neuroprotection and the side effects of HBO as a result of high barometric pressures and long duration.

2. Effect of HBO in stroke models

2.1. Neonatal hypoxia–ischemia

2.1.1. Overview of neonatal hypoxia–ischemia

Hypoxia–ischemia is a common cause of brain injury in the perinatal period. It is thought to be the single most significant contributor to static encephalopathies in children, resulting in mental impairment, seizures, and permanent motor deficits, such as cerebral palsy. Studies show that 20% of all term infants experience ischemic asphyxia, and 20–50% of asphyxiated neonates who experience a hypoxia–ischemia insult expire during the newborn period. Of those who survived, 25% exhibit some level of permanent neuropsychological handicap. Hypoxia is one of the major pathological factors that induce neuronal cell injury, neurodegeneration, and cell death, unbalanced intracellular Ca2+ homeostasis, followed by a cascade of potentially hazardous cellular challenges such as excitatory amino acid toxicity, glycopenia, and acidosis. It often acts in combination with ischemia due to a serious impairment in blood supply of brain tissue caused either by local or general circulatory failure. With ischemia occurring alongside hypoxia (termed hypoxia–ischemia), the severity of the outcome of the hypoxic event increases and the chance of survival of the affected neurons decreases.

One of the primary setbacks to the brain after a hypoxia–ischemia insult is the reduction of oxygen delivery to the tissue. Administration of 100% oxygen under increased ambient pressure is a potent means of increasing the amount of oxygen dissolved in blood plasma, thereby potentially increasing oxygen delivery to the brain. HBO has been shown to be effective in the laboratory, none have been approved in the clinical arena as effective treatments.

2.1.2. Effect of HBO in neonates

Despite the critical, clinical, and socio-economic consequences of perinatal brain damage, no effective clinically therapeutic strategy has been developed. With better understanding of the mechanisms that underlie neuronal cell death, several diverse possibilities have presented themselves for pharmacological intervention. Previous studies have focused on the administration of oxygen and nitric oxide inhibitors, glutamate antagonists, calcium antagonists, potassium channel agonists, nitric oxide inhibitors, glutamate antagonists, calcium antagonists, potassium channel agonists, growth factors, anti-cytokines, and hypothermia. Although many of these studies have been shown to be effective in the laboratory, none have been approved in the clinical arena as effective treatments.

Early application of HBO in neonates within a narrow therapeutic window might prevent or attenuate neurological injury and offer a reasonable and viable alternative. Limited literature available in perinatal or neonatal care provides only for a slim view of the effectiveness and safety of HBO in diseases other than neonatal hypoxia–ischemia. For example, HBO was used in the treatment of necrotizing fasciitis of the abdominal wall in four newborns with two surviving, whereas the other three infants without HBO all died.

Another study reported 63 newborns (4 ± 1 days) with hypocojugation neonatal jaundice. Within 5 days of HBO therapy, bilirubin decreased by 40%, enzymatic function of the liver normalized, and mixed acidosis was arrested. When HBO was used in acute carbon monoxide poisoning during pregnancy at 2.5 atm and 100% oxygen for 90 min regimen, all the babies were delivered at term. In addition,
HBO has been a successful treatment of radiation induced bone and soft tissue complications, cyanotic congenital heart disease, as well as in newborns and children with acute CO poisoning [44,45]. We have recently reported the first controlled experimental study using HBO in a neonatal rat model of cerebral hypoxia–ischemia [46].

We used an established 7-day postnatal rat model because of the histologic similarity between the development of the rat’s brain and that of a 32–34-week gestation human fetus or newborn infant [47]. This model has proven to be useful in many studies and is used in the United States and abroad [48]. Briefly, the carotid artery of a rat pup is exposed and ligated. The animal is then subjected to a period of hypoxia at normal atmospheric pressure (8% O2 for 2 h). This model yields a reproducible pattern of isolated ipsilateral hemisphere injury just distal to the ligated carotid artery. Furthermore, it allows for assessment of mechanisms of brain injury and testing of neuroprotective agents and strategies [49]. Animals that experienced hypoxia–ischemia showed retardation in brain growth, especially in the hemisphere ipsilateral to the ligated artery. Similar to other studies [40], we have shown that severe tissue loss and atrophy accompanied hypoxia–ischemia and led to reduction in brain development using this model [46,50]. However, animals treated with HBO at 3 atm absolute (ATA) pressure for 1 h experienced less brain damage, as represented by improved brain morphology (Fig. 1) and brain weight. Fig. 1 demonstrated photos of brains taken at 24 h to 6 weeks after hypoxia insult. The size of the brain doubled over 6 weeks in normal pups, while only half of the ipsilateral hemisphere remained at 6 weeks in hypoxia–ischemia insult pups. HBO treatment largely prevented the brain atrophy in hypoxia–ischemia pups. Light and electron microscopy demonstrated neurons as being spared following treatment with HBO [46]. Morphology of the brain slides confirmed the results from the photographs that more brain tissues are preserved after HBO treatment (Fig. 2).

When functional status was clinically assessed, it was found that animals that had experienced hypoxia–ischemia scored worse in the postural reflex test than the control animals [46], as previously reported [51]. In contrast, animals that were treated with HBO had similar scores to normal control animals. The finding that in most cases, HBO is able to prevent the sensorimotor deficits caused by hypoxia–ischemia in neonates is in agreement with a previous study that used adult rats [52], suggesting that HBO may provide similar neuroprotection in reducing brain injury and improving neurological outcome. Our observation in neonates is consistent with most other reports that utilized adult animals [53–55] that point to HBO’s ability to reduce behavioral deficits, infarction volumes, and edema, which all ultimately lead to an improved outcome.

In summary, the results of these earlier studies suggest that HBO is able to attenuate the effects of hypoxia–ischemia on the neonatal brain by reducing the progression of neu-

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Fig. 1. Brain atrophy and effect of HBO. Photos of normal rat pup’s brain are shown on the top line. The size of the brain doubled over 6-week time. Hypoxia–ischemia (HI) injury ipsilateral hemisphere, initially shown as brain edema at 48–72 h (arrows), followed by brain atrophy from 1 to 6-weeks (arrow heads). HBO (HI + HBO) at 3 ATA for 1 h reduced brain edema and prevented brain atrophy.
ronal injury and increasing sensorimotor function. Since HBO has been used to treat humans in the past with a certain degree of success, and since it is currently being used as an effective treatment in infants with various disorders, it may provide an effective strategy for the prevention of numerous neurological handicaps that plague many children.

2.1.3. Side effect of HBO in neonates

Concerns regarding the toxicity of oxygen that can occur while HBO is administered at high pressures or over long duration will be discussed in the section of focal cerebral ischemia, since relatively low level of HBO pressures are intended in the treatment of newborns. However, a unique phenomenon regarding HBO in neonates that needs to be addressed is that of retinopathy of prematurity.

2.1.3.1. Retinopathy of prematurity

The emergence of retinopathy of prematurity as a leading cause of blindness in infants has occurred over the past 60 years, seemingly as a result of the advances in neonatal intensive care practices that allowed for the survival of premature infants having significant immature retinal vasculature [56,57]. The incidence for threshold of retinopathy of prematurity (disease progression to the point of necessitating peripheral retinal ablation therapy) for premature infants weighing <1.25 kg is roughly 5%, with about 20–30% of these infants becoming blind despite treatment [58]. Despite vast improvements that have been made in neonatal intensive care practices, the incidence of retinopathy of prematurity has been steady over the last two decades [59]. The idea that elevated levels of oxygen causes retinopathy of prematurity is the main reason why physicians are hesitant about treating hypoxic or premature infants with normobaric or hyperbaric oxygen. This stigma regarding supplemental oxygen has been around since the early 1950s [60,61] when, for lack of a better explanation, it was thought that the oxygen used to treat the infants was causing the blindness, even though retinopathy of prematurity also occur in term and preterm infants not exposed to elevated levels of oxygen [62]. Oxidant stress appears to play a role in the retinal vasoobliteration associated with retinopathy of prematurity [63]. Exposure to hyperoxia affects developing retinas by leading to microvascular degeneration which produces inner retinal hypoxia, which in turn leads to structural and functional changes. These changes can lead to abnormal vascularization resulting in the development of vision-threatening retinopathy [63]. If supplemental oxygen therapy (pressurized or not) is to be used in the treatment of premature or hypoxic infants, questions concerning the safety of hyperoxia, especially in regards to the retina, need to be addressed.

2.1.3.2. Transient HBO does not cause retinopathy

We tested the hypothesis that a single exposure to 100% oxygen for 1 h at various pressures (1, 1.5, and 3.0 ATA) would not produce retinopathy of prematurity in newborn rats. Our results using the same neonatal hypoxia-ischemia rat model demonstrated that hyperoxia at pressures ranging from 1.0 ATA to 3 ATA is neuroprotective when administered after a hypoxic–ischemic insult and that hyperoxia, as used in this
Fig. 3. Retina and HBO. Slides of retina from normal pups are shown on the right side at different magnifications. The top picture shows distribution of arteries (A), veins (V), and capillaries (C). Higher magnifications in the middle pictures show the density of capillaries. The bottom picture shows the morphology of retina with different anatomical layers of cells. Two weeks after treatment with HBO, the density of vasculatures and layer of cells remain the same as in control animals.

study, does not cause the structural changes or abnormal vascularization in the retina that are associated with retinopathy of prematurity [64]. Fig. 3 showed morphological studies of the retina from normal control and from HBO-treated pups at 2 weeks after HBO. No major differences were observed between the two groups upon the density of arterioles, veins, and capillaries.

One of the most sensitive markers for morphological changes in retinopathy is the thickness of the outer plexiform layers (OPL). The OPL is the retinal layer where synaptic contacts between the photoreceptors and second order neurons occur and its formation in rats begins around unclear (P5 and commences on P12) [65]. Exposure to hyperoxia during the first 14 days of postnatal life in rat pups can prevent the normal development of the OPL and result in long-term effects that can eventually impair vision [65,66]. Dembinska et al. [66] have shown that a progressive increase in the duration of hyperoxia causes a gradual thinning of the OPL. In our study, a single exposure to hyperoxia at normobaric and hyperbaric pressures did not result in the thinning of the
OPL, suggesting that this short exposure does not produce the structural anomalies in the retina that are associated with prolonged hyperoxia exposure [64]. Our results, however, do not rule out the possibility of retinal changes induced by high pressure during prolonged duration of treatment. Torbati et al. [67] have shown that retinopathy of prematurity can develop in newborn rats exposed to 100% oxygen at 5 ATA for 5 h, echoed by our observation [64]. Increased ambient pressure can constrict the choriocapillaries and reduce the amount of oxygen transported from the choroid to the inner retina during the period of hyperoxia. The vasoconstrictive response will vary with the degree of hyperbarism, so that at high pressures, constriction may cause a severe and prolonged reduction in choroidal and retinal blood flow. Upon returning to room air from exposure to low levels of hyperbarism, the oxygen levels will be reduced and the stimulus for vasoproliferation will be decreased [68]. In contrast, upon returning to room air from exposure to higher levels of hyperbarism, oxidative damage created by hypoxia-ischemia can lead to the induction of retinal vasoproliferation [67]. These results suggest that it is the duration of the exposure that is important when hyperoxia is given at normobaric pressure, that the pressure is important when hyperoxia is given at hyperbaric pressures, and finally, that hyperoxic exposures can be safely administered at the appropriate pressure and duration. Thus, a single 1 h application of oxygen, either normobaric or hyperbaric, appears to be a safe treatment protocol for neonates after a hypoxia–ischemia insult [64]. The results obtained in this study may help to open the door for the use of hyperoxia therapy for the treatment of hypoxic newborn infants since both normobaric oxygen [69,70] and hyperbaric oxygen [46] have been shown to be effective in neonate and adult models of stroke. However, a problem with hyperbaric oxygenation is increased respiration and arterial hypocapnia, both will affect the cerebral circulation and perhaps arterial blood pressure. It is clear that we do not know what happens to neonatal rodents in terms of arterial gases and blood pressure with hyperbaric oxygenation.

2.2. Focal cerebral ischemia

2.2.1. Overview of focal cerebral ischemia and HBO

An investigation from the American Heart Association indicates that more than 700,000 cases of stroke occur each year in the United States. Among them, 80% have focal cerebral ischemia, either due to thromboses or embolism, as a result of interruption of blood flow to brain tissues. During focal ischemia, the ischemic core is thought to be surrounded by an area that is viable yet non-functioning, a so-called “ischemic penumbra”, from which neuronal cells might be salvaged with adequate therapy [71]. In this area, one of the primary factors causing neuronal damage is energy failure attributable to hypoxia, which causes unregulated calcium influx and subsequently activates enzymes such as phospholipase, protease, and endonuclease, leading to arachidonic acid accumulation, collapse of cytoskeletal elements, and apoptosis [71]. If oxygen supply to the ischemic penumbra can be restored, the cascade of ischemic neuronal damage might be interrupted [72]. However, there are no effective therapies to restore blood supply and oxygen to the ischemic areas after a focal cerebral ischemia. Although thrombolytic therapy has been reported to be effective in a select subset of stroke patients, the limited therapeutic window allowed for only a small percentage of patients to be treated, since there is a risk of possible transformation to a harmful hemorrhage [73]. In this regard, HBO may serve as an important adjunct or alternative means to increase the amount of oxygen physically dissolved in the blood plasma, thereby increasing oxygen delivery to the brain [74].

HBO has been used in the treatment of focal cerebral ischemia in animal models and in human subjects since 1963, when a small pioneer study performed by Jacobson found no benefit of HBO at 2.0 ATA after permanent occlusion of the middle cerebral artery (MCA) in dogs [75]. While the effect of HBO in permanent ischemia is debatable, more controversial data are reported regarding the effectiveness of HBO in cerebral ischemia with reperfusion [29]. Some laboratory studies reported improved outcome [53–55,76–84] while others failed to show any benefit [87,88]. A detailed review of these experimental studies suggests that methodological shortcomings, conceptual problems, and differences in experimental design might account for these discrepancies [53].

2.2.2. Therapeutic window for HBO therapy

The most important factor determining the effectiveness of HBO in ischemic stroke is the therapeutic window for treatment. The primary supposition of HBO therapy in stroke treatment is not to treat the ischemic core but the surrounding areas that are viable yet non-functioning, where residual viable neuronal cells might be salvaged [71]. Therefore, the therapeutic window for intervention with HBO should be similar to that for thrombolytic therapy, with a window of 3–6 h when the ischemic neuronal tissues can still be saved.

We have studied the therapeutic window of HBO in focal ischemic stroke in an established MCA occlusion/reperfusion rat model [52]. After 2 h of occlusion, reperfusion was allowed, and the rats were placed in the HBO chamber. HBO at 3 ATA for 1 h was administered at 3, 6, 12, and 23 h after reperfusion. The rats were sacrificed at 24 h after reperfusion and the infarct volume and neurological function were evaluated. The results showed that the percentage of infarct volume was significantly decreased in both the 3 h (7.9%) and 6 h (5.35%) HBO treatment groups when compared with the control group (11.15%). However, the percentage infarct volume was significantly increased in both the 12 h (23%) and 23 h (17.3%) treatment groups. This study suggests that HBO has a dual effect on the cerebral infarction in MCA occlusion/reperfusion in rats and that the therapeutic window for treatment should be confined to 6 h after the onset of a stroke [52]. This observation was also reconfirmed by a recent report [5].
2.2.3. Single versus multiple treatments

Another important factor to consider when HBO is used as a stroke treatment is the issue of multiple treatments. In most clinical studies, the application of HBO was delayed up to 24 h but multiple applications were used [86,88]. On the other hand, most animal studies utilized one treatment within 3 h of ischemia/reperfusion [7,20,53–55,69,70,76–79,81,82,84,85,89–91]. Very few studies reported on the multiple HBO treatments in animals [54,81,82]. Thus, when comparing the differences between the clinical approach and the experimental protocol, it would seem that multiple treatments may be beneficial even if it is used after a prolonged time following ischemic stroke.

As mentioned above, the known therapeutic window of HBO application was set within 6 h after ischemia/reperfusion [52]. This narrow therapeutic window limits the potential use of HBO in clinical practice for two major reasons. First of all, many patients arrived at the hospital much later than the therapeutic window. In addition, even if these patients are admitted into the hospital within the 3–6 h after the onset of stroke, most of them will be initiated thrombolytic therapy [73]. Thus, when comparing the differences between the clinical approach and the experimental protocol, it would seem that multiple treatments may be beneficial even if it is used after a prolonged time following ischemic stroke.

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We found that delayed, but repeated HBO treatments (with an average of total of seven treatments) have long-term protective effects against ischemic brain damage in rats with regards to neurological outcome and histopathological observations [145]. Delayed HBO treatment, especially at 6 h, significantly decreased cerebral infarct size and improved behavioral outcome in MCA occlusion/reperfusion rats. Delayed, multiple treatments at 24 h had a significant effect on cerebral infarct and neurological functional improvement 4 weeks after ischemia/reperfusion. Fig. 4 demonstrated TTC staining of brain slides, showing evidence of infarction (white color) after MCA occlusion/reperfusion at 1 and 4 weeks. Multiple HBO treatments initiated either at 6 or 24 h reduced infarction at 1 and 4 weeks. This study indicated that the delayed use of multiple HBO treatments, even 24 h after ischemia/reperfusion, improved clinical outcome by reducing the extent of cerebral infarction. Therefore, HBO could be an alternative therapy in the clinical management of acute stroke, particularly in a patient population that is not suitable for thrombolytic treatment due to late hospital admittance.

From these results, it is possible that, with multiple repeated HBO treatments, one might be able to expand the therapeutic window from 6 to 24 h after ischemia/reperfusion, since delayed, but multiple applications of HBO 24 h after ischemia/reperfusion accelerated neurological functional improvement when tested at 4 weeks [145]. Furthermore, this preliminary observation seems to demonstrate that delayed multiple HBO treatments at 24 h did not aggravate brain dam-
age. Therefore, even though a single, delayed treatment with HBO may enhanced cerebral infarction [52], multiple treatments improve neurological function at long-term evaluation. It appeared that the initial harmful effect of delayed application within 24 h was corrected and abolished due to the adaptive effect generated by the multiple HBO applications [93,94].

2.2.4. Side effects

The side effects of pressured oxygen therapy have been noted, especially for high pressure (> 3 ATA) and long duration. It has been shown that HBO (4.96 ATA for 1 h) administered to rats can cause CNS toxicity [95]. A previous study has shown that exposing rats to 4 ATA at 100% oxygen for 90 min was associated with an increased level of lipid peroxidation product, and altered enzymatic anti-oxidation (glutathione peroxidase) in the brain [96]. High levels of HBO reduced cerebral blood flow, possibly by reducing nitric oxide synthase [97]. Chavko et al. [98] stated that 100% O2 at 5 ATA induced seizures. Long duration of HBO at high pressure levels results in adverse effects due to the onset of oxygen toxicity as manifested by the induction of lipid peroxidation and seizures [29].

At lower pressures, Mink and Dutka [91] found that HBO (2.8 ATA for 75 min) was not associated with an increase of lipid peroxidation or with a reduction of neurophysiological recovery, despite increased amounts of oxygen free radicals in the brain, as demonstrated in a global cerebral ischemic rabbit model. The authors suggested that HBO applied immediately after global cerebral ischemia does not promote early brain injury. This report is supported by other studies using similar animal models, that in HBO provided neuroprotection without enhancing lipid peroxidation [76,99]. Sunami et al. [76] used a model of permanent MCA occlusion and exposed the rats (within 10 min of the onset of ischemia) to HBO (3 ATA, 100% oxygen) for 120 min. PaO2 increased 10-fold from 18.53 to 209.41 kPa without impacting or reducing cerebral blood flow at the edge of the ischemic territory. This large increase in arterial oxygen content was achieved by increasing the plasma’s dissolved oxygen rather than hemoglobin-bound oxygen. Despite the increase in oxygen tension, there was no increase in the products of lipid peroxidation [76]. Thus, lipid peroxidation is produced by HBO at pressures higher than 4 ATA [100,101] but not by pressure less than 3 ATA [91,102]. Therefore, the Committee of the Undersea and Hyperbaric Medical Society recommends that a treatment pressure only from 2.4 to 3.0 ATA should be used at the lowest effective pressure to avoid O2 convulsions.

Even though the above-mentioned results are encouraging, HBO may still produce certain adverse effects on the central nervous system. HBO reduces cerebral blood flow by 20–30% in the normal brain via cerebral vasoconstriction [74,20], which might be the consequence of spontaneous hyperventilation attributable to regional cerebral acidosis [103]. The combination of reperfusion and HBO might then induce a free radical load in the ischemic penumbra in theory, even though it was not the case in a recent study using a focal cerebral ischemic rat model [99]. Nevertheless, the combination of HBO and free radical scavengers in acute stroke may have the potential of enhancing the beneficial effects of HBO without suffering its consequential harmful effects.

2.3. Global cerebral ischemia

2.3.1. Overview of global cerebral ischemia

Global ischemia after cardiac arrest, anesthetia accident during surgery, obstruction of airway, drug intoxication or hemorrhagic shock are some of the major causes of brain injury resulting in severe neurological and neurobehavioral deficit [104–107]. Aggressive and selective treatment strategies for global ischemia have been developed over the past years such as the anti-excitotoxicity, free radical scavengers, prevention of neuroinflammation, and inhibition of apoptosis. These strategies have all achieved limited success, however, primarily because of an incomplete understanding of the mechanisms of neuronal death after global ischemia [106]. Hypothermia, which targets multiple molecular pathways, offers significant brain protection in animal models and in patients after cardiac arrest [108,109]. HBO, another pan-brain protective therapy, increases oxygen delivery to the ischemic brain, rescues neuronal tissues, preserves the functional activity of the brain, and attenuates the "secondary brain injury" in animals [12,19,46,53,54,110,111], including in the global ischemia animal models [4,20,79,82,112,113].

2.3.2. Effect of HBO on global ischemia

The most vulnerable brain region after global ischemia is the hippocampus where neuronal death, especially apoptotic death, leads to impairment of neurological and neurobehavioral function. Due to the shortage of effective neuroprotective therapies, HBO is an extremely attractive alternative for the treatment of global ischemia, especially when caused by cardiac arrest, anesthesia accident during surgery, drowning, electric shock, obstruction of airway, drug intoxication, or hemorrhagic shock.

In an established four-vessel rat occlusion model, survival time and rate after global ischemia were recorded for 14 days [79]. All animals died in the no treatment group, while 45% rats survived after treatment with HBO (3 ATA, 1 h). When compared to control animals that did not receive any HBO, even the HBO-treated animals that did not survive the entire 14-day period still notably lived longer (59.8 ± 9.1 h versus 17.9 ± 2.7 h). Similar observation was made in a canine global ischemic model [82]. Fifteen minutes of complete global cerebral ischemia was achieved by occlusion of the ascending aorta and the caval veins. HBO increased survival rate and improved neurological function. A combination of HBO with nicardipine achieved similar results [81]. Post-ischemic HBO was tested after experimental cardiac arrest and resuscitation and found to inhibit neuronal death and improve neurological outcome [4]. In a global ischemic rat
model with hypotension (by way of exsanguination), HBO reduced cell death in the hippocampal regions [113]. Fig. 5 demonstrated Nissl staining of hippocampus and cortex in rats at 96 h after global ischemia–hypotension. Most neurons in CA1 region died as shown by lacking of Nissl staining. Many neurons showed sign of damages in the cortex with condensed nuclei. HBO (3 ATA, 2 h) applied at 1 h after global ischemia–hypotension prevented a significant number of neuronal cell death in the CA1 and the cortex.

The treatment protocols for global ischemia, to include the therapeutic window, multiple applications, and low pressure of HBO within 3 atm, are all similar to those of focal cerebral ischemia.

3. Mechanisms of HBO in stroke

The mechanism of HBO-induced neuroprotection includes enhancing of neuronal viability via an increase of tissue oxygen delivery to the area of diminished flow, reducing brain edema, and improving post-ischemia metabolism [29]. The major factor precluding neurological recovery after ischemia is reperfusion failure. The dissolved oxygen provided by HBO could “reperfuse” the ischemic area due to the greater pressure gradient, even in the presence of reduced cerebral blood flow. In the interim, HBO decreases the deformability of the red blood cells [114,115]. A decrease in the deformability of the impaired red blood cells may help to prevent irreversible neurological injury [115,116]. Additionally, there are other effects of HBO therapy that may contribute to the prevention of permanent neurological injury. Fig. 6 summarizes the general mechanisms of HBO neuroprotection.

3.1. Oxygen supply

One of the major beneficial effects of HBO in ischemic cerebral ischemia is in its ability to improve tissue oxygen delivery [76]. HBO enhances neuronal viability by increasing the amount of dissolved oxygen in the blood without significantly changing blood viscosity. High plasma oxygen concentration in an ischemic episode is important because capillary blood flow during ischemia consist mainly of plasma blood flow [117]. Enhanced oxygen delivery might lead to the improvement of penumbral energy metabolism and, as a result, decrease susceptibility to additional metabolic challenges, such as peri-infarct depolarization [71,72]. In addition, HBO reduces brain edema, reduces brain vascular permeability, and enhances blood–brain barrier integrity [20]. HBO also restores ion pump function, improves post-ischemic cerebral metabolism, and allows time for collateral circulation to develop [4,30]. Furthermore, by raising the tissue PO2, HBO might initiate a cellular and vascular repair mechanism. When used after radiation injury, HBO has been shown to increase tissue oxygen concentration, thereby stimulating angiogenesis and establishing a new capillary blood supply [118]. In contrary, a recent study indicated that HBO improved cerebral oxygen extraction ratio but failed to enhance oxygen delivery and metabolic rate for oxygen [4].

3.2. Cerebral metabolism

It has been previously demonstrated that biochemical mechanisms are involved in the development of irreversible ischemic brain injury. Excessive lactic acidosis, high intracellular calcium levels, formation of free radicals, and high...
concentrations of excitatory amino acids are associated with ischemic neuronal damage [71].

Fig. 6. Mechanisms of HBO neuroprotection. Cerebral hypoxia-ischemia disables energy metabolism, reduces ATP production, releases glutamate, and causes calcium overload and depolarization. Mitochondrial damage follows, with oxygen radical generation and inflammatory reactions. All these pathological events not only lead to apoptotic neuron death, but also result in brain infarction, brain edema and the dysfunction of blood–brain barrier. The final outcome is the death or disability of patients. HBO either improves oxygen delivery or oxygen extraction to enhance neuronal viability. HBO protects the blood–brain barrier and reduces cerebral edema. Cerebral metabolism is improved by HBO and levels of glutamate, glucose, and pyruvate are stabilized. The inhibitory effect of HBO on inflammatory agents and on apoptosis may be mediated by the re-regulation of superoxide dismutase and by enhancing the expression of pro-survival Bcl-2 genes. Finally, HBO decreases the deformability of the red blood cells to improve microcirculation and reduce hypoxia–ischemia.

Fluctuations in the extracellular fluid levels of energy-related substances such as glucose, lactate, pyruvate, and excitatory amino acids (i.e., glutamate) reflect intracellular metabolic disturbances produced by ischemia [71]. HBO might increase oxygen concentration, enhance neuronal viability, and initiate cellular and vascular repair [20,76,118]. These factors could partially restore the neurochemical uptake mechanism and increase their storage in astrocytes. For example, HBO decreased glucose, pyruvate, and glutamate level from ischemic penumbra almost to the control level (pre-occlusion level) [19]. This study suggests that regulation of these striatal metabolites by HBO may partially reduce cerebral infarction. In addition, HBO enhances hippocampal superoxide dismutase and preserves Na⁺,K⁺-ATPase activities [112]. The mechanism of HBO-induced neuroprotection in focal cerebral ischemia was suggested, at least in part, to be a result of a reduced level of extracellular dopamine [119]. The effect of HBO on improvement of brain metabolism has also been observed in these patients [12,120].

3.3. Inflammation

Several studies suggest that HBO may protect the brain from ischemic damage by inhibiting the inflammatory process [121–123]. After cerebral ischemia, an infiltration of the affected brain by inflammatory cells contributes to the progression of cerebral ischemic damage [124]. In peripheral organs, such as in a rat’s intestinal ischemia–reperfusion model, HBO reduced leuko-sequestration and neutrophil pre-activation. The percentage of NBT-positive cells increased in all of the animals after reperfusion, but the increase was significantly reduced by HBO treatment [123]. Weisz et al. reported that HBO treatment in Crohn’s disease (a perianal inflammatory bowel disease) decreased TNF-α, IL-1, and IL-6 secretion by circulating monocytes [122]. Exposure to HBO (3 ATA, 45 min) inhibited the carbon monoxide-mediated adherence of leukocytes in brain microvessels due to $\beta_2$ integrins [121]. Another inflammatory factor which is normally expressed in neurons of the brain in adult animals is the cyclo-oxygenase-2 (COX-2). Up-regulation of COX-2 expression occurs after inflammatory stimuli, including in cerebral ischemia and hypoxia [125,126]. HBO reduced COX-2 mRNA and protein expression in ischemic hemispheres after MCA occlusion/reperfusion in rats [22]. In addition, a recent study indicates that cellular protection of HBO is associated with diminished infiltration of polymorphonuclear neutrophils into the injured brain [127]. HBO reduces the ischemia-induced down-regulation of the neurotrophin-3 mRNA level at 4 h post-ischemia, and significantly increases cell survival 7 days after reperfusion, suggesting that HBO can maintain the neurotrophin-3 mRNA level in the hippocampus, and thus, be beneficial to the ischemic brain when administered within a certain time frame [128].
3.4. Apoptosis

Cell death, either by necrosis or apoptosis, occurs in the brain tissues during the first few days after cerebral ischemia [129]. Necrotic cell death is characterized by cellular swelling, nuclear pyknosis with karyorrhexis, and cytoplasmic eosinophilia. Apoptosis is characterized by morphological and biochemical features, including cell shrinkage, formation of apoptotic bodies, and extensive inter-nucleosomal fragmentation. Cerebral hypoxia-ischemia produces a cascade of interconnected pathological processes, including changes in intracellular calcium, excitatory amino acid, oxidative stress, and inflammatory response, leading to apoptosis in the ischemic penumbra [71]. Prevention of apoptosis becomes a therapeutic strategy to preserve brain tissues and promote functional recovery [130].

One of the most important mechanisms of HBO neuroprotection is the inhibition of apoptosis in injured brain tissues. HBO reduces brain injury in neonatal hypoxia-ischemia by inhibition of apoptosis [23], in focal cerebral ischemia [24], and in global cerebral ischemia [113]. Multiple analytic methods were used and consistent results demonstrated that HBO decreased the activity and expression of caspase-3, reduced PARP cleavage, and abolished DNA fragmentation. It is likely that inhibition of apoptosis by HBO translates into reduction of cerebral infarction and brain tissue preservation [23,24].

The mechanism responsible for HBO-induced anti-apoptotic effect is not clear, although several plausible explanations have been proposed. The first likely mechanism may be that, by increasing oxygen delivery to an area with diminished blood flow, HBO counteracts hypoxia and reduces brain injury. Secondly, by reducing hypoxia-ischemia, HBO reduces all the pathological events as a consequence of hypoxia, including brain edema, increased blood-brain barrier permeability, post-ischemia derangement of brain metabolism [19], and inflammation [22,131]. Thirdly, HBO may directly affect gene expression in those that are sensitive to oxygen or hypoxia. Our recent observation has shown that HBO decreases hypoxia-inducible factor-1α (HIF-1α) and multiple other genes related to apoptosis [146]. Fig. 7 showed a schematic cascade of apoptotic cell death after hypoxia-ischemia. Deprivation of oxygen during hypoxia-ischemia triggers the accumulation of HIF-1α, which in turn activates its target genes including the genes for angiogenesis (VEGF), erythropoiesis (erythropoietin), and glycolysis (glycolytic enzymes and glucose transporters) (not shown in Fig. 7). In addition, HIF-1α activates tumor suppressor gene p53, Nip3 from the pro-apoptotic Bcl-2 family, and caspase-9, all of which have strong association to apoptosis. Activation of p53 leads to release of cytochrome C and the activation of caspase-9 and caspase-3, resulting in cell death, especially by apoptosis. Several studies also reported that HIF-1α is involved in neuronal death after brain injury.

![Fig. 7. Apoptosis and HBO. Oxygen depletion leads to the stabilization of hypoxia-inducible factor-1α (HIF-1α) in cells. HIF-1α binds with tumor suppressor p53 or other apoptotic genes. Released cytochrome c activates caspase-9 and caspase-3 leads to cell death. HBO restores oxygenation, improves energy levels, and interrupts this cell death pathway.](image-url)

3.5. Plasticity

Following the initial cerebral ischemia, post-acute brain plasticity is an important mechanism for determination of functional brain improvement [132,133]. The absence of neuroanatomical plasticity following brain trauma or hypoxia-ischemia is attributable to several factors, including glial scars, lack of neurotrophins, and growth inhibitory molecules [134]. Nogo-A is one of the most powerful growth inhibitors among these myelin-associated inhibitors [135,136]. Nogo-A binds to the Nogo receptor (Ng-R) and activates intracellular Rho GTPase signal pathways [137]. An enhanced activity of the Nogo-A pathways might interfere with CNS plasticity and hamper the neurological functional improvement. IN-1 (a Nogo-A antibody) and NEP1-40 (an antagonist of Ng-R) treatments attenuated spinal cord lesions [136] and improved stroke outcome [132,134].

We have studied the effect of HBO on the expression of Nogo-A, Ng-R, and RhoA in the ischemic brain cortex following global ischemia. Transient global ischemia (10 min) produced remarkable brain cell loss at 96 h and 7 days. Accompanying the morphological injuries is an immediate elevation in the expression of Nogo-A, Ng-R, and their downstream effector RhoA, present in the ischemic cerebral cortex HBO has been found to reduce their expressions [113]. An interesting observation is that all factors in the Nogo-A pathways responded immediately, increased within 6 h, and peaked around 48 h after global ischemia. This observation is surprising, considering that the function of the
Nogo-A system during the post-acute regeneration phase is that of growth inhibition. Therefore, it would appear that the mechanism for neuronal plasticity begins during the acute stage after global ischemia. Indeed, Nogo-A plays an important role in preventing regeneration immediately after injury, and before glial scar formation [132]. Early application of a Nogo-A antibody within 24 h improved behavior and neuroanatomical plasticity after experimental stroke [132]. These results indicate that suppression of Nogo-A pathways by HBO might partially contribute to the improvement of the neurological function observed previously in global ischemic animal models [4,20,79,82,112].

3.6. Ischemic tolerance

An extremely promising area of HBO application is ischemic tolerance or ischemic preconditioning. Non-lethal stimulations, including HBO, induce ischemic tolerance which may decrease brain injury caused by lethal stimulations. Even though the role of ischemic tolerance in acute stroke is debatable, HBO has been tested to produce ischemic tolerance in stroke models. In a rat MCA occlusion/reperfusion model, animals received 1 h HBO at 2.5 atm absolute in 100% oxygen every day for 3–5 days before conducting ischemic surgery. Preconditioned rats had a much better neurological outcome, with decreased infarct volume [25]. Similar HBO neuroprotection was induced in mice, which is, however, strain dependent [138]. HBO ischemic tolerance has also been demonstrated in a rabbit model of spinal cord injury [139].

The mechanisms of HBO ischemic tolerance are related to the increases of Bcl-2 and manganese superoxide dismutase activities in the ischemic brain [140]. Elevation of Bcl-2 protects brain cells and reduces apoptosis [129]. Induction of superoxide dismutase may be related to the oxidative action of HBO [141]. In addition, HBO induces catalase induction which may contribute to HBO ischemic tolerance as shown in the heart [142]. HBO also enhanced the expression of heat shock protein 72 in ischemic brain tissues and reduced cell death [83]. The role of heat shock proteins in ischemic tolerance has been established [143].

4. Perspective

Two obstacles that hamper the extensive use of HBO in stroke treatment are the lack of controlled clinical trials and limited knowledge of established mechanisms. Misunderstanding of the toxicity of HBO also retards its clinical application, especially in infants or children. Understanding of the toxicity of HBO also retards its clinical application, especially in infants or children. Therefore, careful and controlled clinical trials and further elucidation of its fundamental neuroprotective mechanisms are needed from both clinical and basic science studies before HBO can be considered as a routine stroke treatment. The extreme HBO toxicity in stroke treatment is generated by high pressures (>3 ATA) and prolonged duration, which are generally not used in routine treatment protocols. The possibility of retinopathy of prematurity, as shown in this paper, is limited if low pressure and short duration of HBO is applied. In future clinical trials, several important factors need to be considered: therapeutic window, optimal pressure, and duration of HBO treatment. In addition, one needs to be aware that lower pressure [144], and at times, even normobaric pressure [69,70] can be neuroprotective, at least in animals.

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