A review of oxygen therapy in ischemic stroke

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Neuroprotective drugs have so far failed clinical trials, at high cost, and intravenous tissue plasminogen activator (i.v. tPA) remains the only FDA-approved acute stroke therapy. Hyperoxia, acting via a multiple direct and indirect mechanisms, may be a powerful neuroprotective strategy to salvage acutely ischemic brain tissue and extend the time window for acute stroke treatment. Of the available oxygen delivery methods, hyperbaric oxygen therapy (HBO) appears to be the most potent, while even normobaric oxygen therapy (NBO) may be effective if started promptly after stroke onset. HBO has so far failed to show efficacy in three clinical trials. The failure of these trials is probably attributable to factors such as delayed time to therapy, inadequate sample size and use of excessive chamber pressures. Previous trials did not assess long-term benefit in patients with tissue reperfusion. In this modern era of stroke thrombolysis and advanced neuroimaging, oxygen therapy may have renewed significance. If applied within the first few hours after stroke onset or in patients with imaging evidence of salvageable brain tissue, oxygen therapy could be used to ‘buy time’ for the administration of thrombolytic or neuroprotective drugs. This article reviews the history and current rationale for using oxygen therapy in stroke, the mechanisms of action of HBO and the results of animal and human studies of hyperoxia in cerebrovascular diseases. [Neurol Res 2007; 29: 173–183]

Keywords: Hyperbaric oxygen therapy; normobaric oxygen therapy; neuroprotection; acute stroke therapy; hypoxic–ischemic encephalopathy

INTRODUCTION

Stroke is the leading cause of long-term disability and the third leading cause of death in the United States, with an annual direct and indirect cost exceeding $56 billion. Intravenous tissue plasminogen activator (i.v. tPA), the thrombolytic agent, is the only Food and Drug Administration (FDA)-approved treatment for acute ischemic stroke. While early thrombolysis is clearly beneficial, it is believed that stroke outcome can also be improved with ‘neuroprotective’ therapies that interrupt one or more of the pathways of ischemic cell death after stroke. At present, the field of stroke therapy is limited by two major problems. First, use of tPA is restricted to less than 5% of stroke patients because of its narrow therapeutic time window (3 hours). Second, no neuroprotective drug has proved effective in phase III human studies despite promise in animal studies. Clearly, a strategy that can both salvage ischemic neurons and safely extend the time window for stroke thrombolysis would be a significant advancement.

Ischemic stroke therapy with hyperbaric oxygen (HBO) has been advocated for decades because it is widely believed that raising oxygen tension within ischemic tissues would reduce ischemic necrosis. However, HBO has so far failed to show efficacy in three clinical trials despite promise in numerous animal studies. A careful analysis of the literature, a thorough understanding of the shortcomings of the previous trials and data from recent animal studies, suggest that supplemental oxygen, in the form of hyperbaric as well as normobaric oxygen therapy (NBO), has tremendous therapeutic potential in this modern era of stroke thrombolysis with advanced neuroimaging capability to select appropriate patients and assess tissue outcomes. Specifically, oxygen therapy could be highly effective in certain clinical situations (e.g. within the first few hours after stroke onset) and could be used to ‘buy time’ for administration of thrombolytic or neuroprotective drugs. In this chapter, we will review the history and current rationale for using oxygen therapy in stroke, discuss the mechanisms of action of HBO and summarize the results of animal and human studies of hyperoxia in focal and global stroke in adults and in neonates.

EVOLUTION OF HBO AS A STROKE THERAPY

Compressed air has been advocated as a medical therapy since the mid-17th century, even before the discovery of oxygen by Priestly in 1775. Junod in 1834 was probably the first to use hyperbaric chambers for medical therapy, and the first mobile hyperbaric operating room was developed in 1877. In the early 20th century, Cunningham operated the largest and only functioning hyperbaric chamber in Cleveland. The medical profession eventually closed down his chamber because he was unable to prove therapeutic efficacy. Until this time, hyperbaric air and not oxygen was used. In 1960, Boerema published a landmark study showing...
that exsanguinated pigs could be survived by placing them in HBO chambers. This study intensified efforts to use HBO to treat organ ischemia. Jacobson and Lawson published a study showing no benefit with HBO at 2.0 ATA after permanent middle cerebral artery occlusion (MCAO) in dogs. Thereafter, numerous animal studies have documented substantial benefit with HBO in animal models of transient and permanent focal stroke, providing a rationale for HBO therapy in stroke. In 1964, Moon and colleagues reported treatment of a patient with coronary thrombosis and atrial fibrillation with HBO. The patient developed hemiparesis on day 2 after his myocardial infarction. He was immediately placed in a HBO chamber and his neurological deficits disappeared, demonstrating for the first time that HBO was effective in hyperacute stroke. Ingvar and Lassen from Lund University published a report on the use of HBO in four patients with stroke. They documented transient reversal of neurological deficits and improvement in electroencephalography (EEG) abnormalities with HBO therapy, and concluded that HBO should be tested on a larger scale. Since then, numerous case reports and case series documenting the use of HBO in stroke have been published, including the largest case series of 122 patients by Neubauer and End. Based on these anecdotal reports, the need for conducting clinical trials of HBO in stroke was recognized.

The results of the first two clinical trials of HBO were published. Unfortunately, both trials reported negative results which, in conjunction with the known difficulties and pressure-related complications of HBO, and the growing fear that hyperoxia could increase the generation of toxic free radicals, led to a period of skepticism about the safety and efficacy of HBO in stroke. Over time, it became clear that factors such as the timing and duration of HBO, the optimal chamber pressure, stroke subtype and optimal sample size, had the timing and duration of HBO, the optimal chamber pressure, stroke subtype and optimal sample size, had been overlooked in the previous clinical trials of HBO. Dr James F. Toole convened a workshop in Winston-Salem, NC, USA, to discuss the role of HBO in stroke and to design a clinical trial of HBO delivered within the first 3 hours after acute ischemic stroke. The participants, all experts in acute stroke therapy or hyperbaric medicine, agreed that HBO had tremendous therapeutic potential given the extensive safety and efficacy data from animal studies. They acknowledged the need for adequately powered and well-designed clinical trials of HBO in acute stroke. A multicenter trial of HBO in stroke is now in the planning stages. In the interim, Rusyniak et al. have published the results of their small study again showing that HBO is ineffective in ischemic stroke. This study is known to have several shortcomings and has raised the level of controversy surrounding HBO in stroke.

Recent animal data indicate that oxygen therapy is the most effective if applied early, for short durations, and that long-term benefit can be maximized if tissue is reperfused. With the advent of stroke thrombolysis and advanced neuroimaging, and newer insights into clinical trial design, there is optimism that oxygen therapy will eventually prove to be an effective therapeutic strategy that can improve stroke outcome either independently or by extending the time window for thrombolysis or neuroprotective drug therapy.

Rationale for Using Oxygen Therapy in Stroke
Ischemic stroke results in regions of variably compromised blood flow and oxygen supply distal to the site of arterial occlusion. The ‘core’ of the ischemic territory refers to regions of severely compromised blood flow where cellular injury is irreversible and tissue is non-salvageable. In such regions, cell death usually occurs within minutes. Surrounding the ‘core’ regions, are areas of reduced blood flow supported by collateral circulation, where tissue is at risk for infarction but still salvageable. This tissue is referred to as the ‘ischemic penumbra’ and constitutes the target for stroke neuroprotective therapies. In humans, the results of positron emission tomography (PET) and functional magnetic resonance imaging (MRI) studies suggest that the ischemic penumbra exists for several hours or more after symptom onset. With passage of time, there is a reduction in the volume of ischemic penumbra and growth of the infarct core. It is believed that hyperoxia, by raising tissue PO2 levels in the ischemic penumbra, can reduce final infarct volume and attenuate stroke-related neurological deficits. In addition, the application of HBO after completed stroke is believed to enhance post-stroke recovery, and treatment before stroke onset may have preconditioning effects.

Hyperoxia is attractive acute stroke therapeutic option because it has several properties of an ‘ideal’ neuroprotective agent. Unlike most pharmaceutical neuroprotective drugs, it easily diffuses across the blood–brain barrier (BBB) to reach target tissues, is simple to administer, well tolerated, can be delivered in 100% concentrations without significant side effects and theoretically can be combined with other acute stroke treatments such as tPA. Further, as discussed below, it is known to act on multiple cell death pathways and may have beneficial hemodynamic effects. HBO therapy has been widely studied because it is the most effective method to increase brain tissue oxygenation. Other methods to increase oxygen delivery (currently under development) include the use of perfluorocarbons, hemoglobin-based oxygen carriers and aqueous oxygen solutions. Our group has recently become interested in the potential therapeutic effects of early HBO therapy or the administration of high-flow oxygen via a facemask. Whether one method of oxygen delivery is superior to the others remains to be determined; at this stage, it appears that the timing of therapy is critical and at equivalent timings, HBO is probably the most potent.

Mechanisms of Action
Basic science research over the last three decades has delineated four fundamental pathways of irreversible cellular injury after stroke: excitotoxicity, oxidative/nitrosative stress, inflammation and apoptosis. Tissue...
hypoxia is a key contributor to these pathways; therefore, it is logical that increasing oxygen levels in ischemic tissues would be neuroprotective. Early studies were based on the rationale that the ten-fold higher plasma concentration of dissolved oxygen achievable with HBO would facilitate oxygen diffusion into ischemic tissues and minimize reliance on hemoglobin-bound oxygen. Recent studies have shown that HBO also acts via multiple indirect biochemical, molecular and hemodynamic mechanisms (Figure 1).

The main effect of HBO is improved brain tissue oxygenation and metabolism in penumbral tissues. In a focal stroke model, HBO significantly increased arterial oxygen pressure and content, resulting in a 20% increase in oxygen supply to the ischemic periphery. In models of traumatic brain injury, HBO increased brain tissue pO2, increased the cerebral metabolic rate of oxygen, decreased brain lactate and pyruvate levels, and improved mitochondrial function. Recent studies indicate that HBO also acts via multiple indirect biochemical, molecular and hemodynamic mechanisms (Figure 1).

Figure 1: 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 pathologic 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 cerebroedema. 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.

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ion pump function. Finally, by increasing oxygen levels, HBO might inhibit post-anoxic depolarization which is known to contribute to ischemic lesion growth.

Several studies have documented that HBO has anti-inflammatory effects. In rodent stroke models, HBO treatment reduced cyclooxygenase-2 mRNA and protein levels, and decreased polymorphonuclear cell infiltration. In models of ischemia/reperfusion injury, HBO has been shown to reduce intercellular adhesion molecule-1 and reduce polymorphonuclear neutrophil adhesion through the induction of endothelial nitric oxide synthase. Recent evidence suggests that HBO inhibits apoptosis, a major mechanism of delayed cell death. In a global ischemia/reperfusion model, HBO decreased the expression of multiple pro-apoptotic genes including hypoxia inducible factor-1alpha, p53, caspase-9 and caspase-3. Similar results were noted in models of focal stroke, neonatal hypoxic–ischemic brain injury, and brain trauma. These anti-inflammatory and anti-apoptotic effects of HBO may help to preserve brain tissue and promote neurological recovery.

HBO decreases blood viscosity, reduces platelet aggregation and improves the microcirculation. A concern with HBO therapy is that it can worsen ischemia by inducing vasoconstriction. However, it is important to understand that local pO2 increases with HBO therapy despite its cerebral blood flow lowering effects, and by inducing vasoconstriction in the surrounding non-ischemic brain, HBO might actually drive blood into the autoregulation-impaired regions of
ischemic brain (the ‘inverse steal’ effect\textsuperscript{34,35}). That NBO may have similar hemodynamic effects is suggested by serial perfusion-MRI findings of recent rodent and human stroke studies\textsuperscript{34,35}. Overall, the neuroprotective, vasoconstrictive and anti-inflammatory effects of HBO are believed to prevent cell death, preserve blood-brain barrier integrity and reduce vascular permeability, all contribute to decreased cytotoxic and vasogenic brain edema\textsuperscript{36}.

While the above effects are applicable to the acute stroke setting, there is some evidence that HBO has ischemic pre-conditioning effects and promotes recovery after stroke. HBO has been shown to increase angiogenesis via its effects on vascular endothelial growth factor, promote cellular and vascular repair\textsuperscript{37}, and inhibit the Nogo-A pathways\textsuperscript{38} which impede brain plasticity. Repeated applications of HBO 3–5 days before spinal cord and brain ischemia have been found to mitigate neuronal loss and reduce neurological deficits, although the effects may be dose- and strain-dependent\textsuperscript{39–41}. Further investigation is warranted to assess the use of HBO in the pre- and post-stroke setting.

**ANIMAL STUDIES OF HBO IN FOCAL ISCHEMIC STROKE**

Over 20 animal studies of HBO in focal stroke have been published. Zhang \textit{et al.}\textsuperscript{42} and Helms \textit{et al.}\textsuperscript{43} have recently summarized these results in excellent review articles. HBO has been studied in models of transient focal stroke\textsuperscript{23,27,28,30,44–52} as well as permanent focal stroke\textsuperscript{16,51,53–57}. The rodent model has been used in most of these studies, with gerbils\textsuperscript{53–55} and cats\textsuperscript{46} being the other species studied. The duration of ischemia has varied from 1 to 6 hours, the range of chamber pressures from 1.5 to 3 ATA and the time to therapy from few minutes to 24 hours after stroke onset\textsuperscript{42}. In general, single sessions have been used, with only few studies investigating the effects of multiple sessions\textsuperscript{44,47,58}. Positive results have been documented in all but three studies\textsuperscript{5,47,57}, providing overwhelming evidence that HBO is efficacious in stroke.

A careful analysis of these studies shows that the efficacy of HBO depends on several variables including the duration of ischemia, timing of therapy, number and duration of HBO exposures, and chamber pressure. In contrast to NBO, HBO has been found beneficial in permanent ischemia\textsuperscript{16,51,53–56}. At least three studies\textsuperscript{46,51,59} have shown that the efficacy of HBO diminishes with longer period of ischemia. In a study by Veltkamp \textit{et al.}\textsuperscript{59}, HBO at 2.5 ATA proved superior to HBO at 1.5 or 1.0 ATA in reducing neurological deficits and infarct volumes after 75 minute transient focal ischemia. These results suggest that HBO’s effects are dose-dependent. However, based on clinical experience, 1.5 ATA is believed to be the optimal pressure in humans\textsuperscript{12}.

Several studies have investigated the therapeutic time window of HBO. Weinstein \textit{et al.} administered HBO at 1.5 ATA for 40 minutes and after 6 hour MCAO, and before, during and after 24 hour MCAO in unanesthetized cats\textsuperscript{46}. Neurological deficits and infarct volumes at day 10 were reduced by 93 and 58%, respectively, with HBO therapy up to 3 hours during 6 and 24 hour MCAO. HBO therapy at hour 4 failed to reduce infarct volume although there was improvement in neurological deficits in the 6 hour MCAO group. Using a rodent 2 hour transient focal stroke model, Zhang’s group showed that HBO (3 ATA for 1 hour) was beneficial if administered 3 and 6 hours but was harmful if administered 12 and 23 hours after ischemia\textsuperscript{59} (Figure 2). Similarly, Lou \textit{et al.} found that HBO (3 ATA for 1 hour) was neuroprotective when initiated within 6 hours, but deleterious when initiated 12 hours after transient MCAO in rodents\textsuperscript{51}. These results suggest that single-session HBO has a short therapeutic time window. However, Zhang’s group has also shown that the time window can be extended with multiple, delayed treatments. Rats treated with HBO daily for 6 days, starting 6 and 24 hours after transient focal stroke, had significantly reduced infarct volumes and neurological deficits as compared to untreated rats\textsuperscript{58}. Supporting the efficacy of multiple treatments is the fact that such regimens also extend the therapeutic time window for acute spinal cord injury\textsuperscript{60}. These findings have major clinical implications because of the widespread delays in hospital arrival after stroke symptom onset.

Recent in vivo DWI studies provide insights on the time course of HBO’s effects in stroke. Schabitz \textit{et al.}\textsuperscript{56} (Figure 3). Treated rats with room air or HBO (2.0 ATA for 1 hour) starting 2 hours after permanent MCAO. DWI and perfusion-weight MRI (PWI) were performed after MCAO, at serial time points before and after HBO therapy. A significant reduction in DWI lesion volumes was observed as early as 5 hours after MCAO. Radiologic and functional benefits persisted at least until 5 days. Veltkamp \textit{et al.}\textsuperscript{23} performed serial MRI at minute 15 and hours 3, 6 and 24 in Wistar rats treated
with HBO at either 3.0 or 1.0 ATA after 40 minute transient focal cerebral ischemia. DWI and T2 lesion volumes, apparent diffusion coefficient (ADC) values and pathologic infarct volumes were attenuated at all time points in rats treated with HBO at 3.0 ATA. Notably, these investigators have previously shown that HBO at 2.5 ATA is superior to HBO at 1.0 ATA. These results indicate that in the transient focal stroke model, oxygen delivery at hyperbaric pressures is more efficacious than NBO therapy.

HBO IN HUMAN ISCHEMIC STROKE

Despite the longstanding interest and abundance of animal studies concerning the effects of HBO in stroke, most human reports have been on isolated cases or case series. To date, more than 2000 stroke cases treated with HBO have been documented in literature. Virtually, all these reports are favorable. In addition to promoting neurological recovery in the acute setting, HBO has been found to reduce the frequency of recurrent strokes, improve recovery after stroke and predict success from cerebral revascularization procedures. The true efficacy or safety of HBO cannot be assessed from these reports because of publication bias and the heterogeneity of the stroke patients studied, the variable timings and doses of HBO used, and the non-uniformity of outcome measurements.

To date, three randomized clinical trials of HBO in stroke have been published. Anderson et al. randomized 39 patients with ischemic stroke to pressurized air or oxygen at 1.5 ATA for 60 minutes every 8 hours to a total of 15 sessions. This trial was interrupted early when an interim analysis showed a trend towards improvement in neurological examination scores and smaller infarct volumes at month 4, in subjects treated with hyperbaric air. While the authors eventually attributed these results to an artifact of the randomization process, they did not resume the trial because of logistical difficulties and poor patient tolerance.

Nighoghossian et al. randomized 34 subjects (including 21 males) with middle cerebral artery stroke to receive either HBO or hyperbaric air within 24 hours after symptom onset. Treatments were administered daily for 40 minutes at 1.5 ATA for 10 days. Therapeutic efficacy was assessed at month 6 and year 1 using the Rankin score,Trouillas score and changes in the Orgogozo scale score. All subjects received standard stroke interventions including heparin and rehabilitation therapy. Seven subjects were withdrawn because of complications. Of the remaining 27 subjects, the Orgogozo and Trouillas scores at year 1 were significantly better in the HBO group; however, a comparison of the pre- and post-therapy differences in the two groups at month 6 and year 1 did not show statistical significance on any scale.

Rusyniak et al. randomized 33 patients (including 22 males) with ischemic stroke <24 hours and National Institutes of Health Stroke Scale (NIHSS) score below 23, to HBO or sham therapy. The HBO group received 100% oxygen at 2.5 ATA, and the sham group received 100% oxygen at 1.14 ATA, both for 60 minutes in a monoplace chamber. There were no differences in 24 hour NIHSS scores between the groups. By 3 months, neurological outcome scores (NIHSS, Rankin scale, Barthel index and Glasgow outcome scale) were better in the sham group compared with the HBO-treated group, reaching statistical significance in all scales except the Barthel index. The authors concluded that HBO offers no benefit and may even be harmful in stroke.
In a recent meta-analysis, Bennett et al. concluded that the use of HBO in stroke could not be justified based on existing data. However, these trials had several shortcomings. The lack of a significant effect may be related to the small number of patients and delays in initiating HBO therapy. In the trial of Anderson et al., HBO was administered up to 2 weeks after stroke onset. CT scan was used to exclude hemorrhage only in the study of Rusyniak et al. The success of blinding was not formally tested in any trial. The use of excessively high chamber pressures (2.5 ATA) in the study of Rusyniak et al. has been criticized; moreover, in this trial, the sham group actually received 100% oxygen and not room air. Because NBO is itself beneficial, the validity of such a sham group has been questioned. Finally, tissue reperfusion status was not assessed in any trial. Future trials should be adequately powered, should focus on early therapy, should use neuroimaging to select appropriate subjects and assess safety (edema and hemorrhage) and efficacy, should select an appropriate HBO dose, pressure and treatment regimen based on evidence from earlier studies, and should use sensitive functional scales. The efficacy of HBO in patients with subsequent tissue reperfusion should be determined, and HBO should be investigated as an adjunct to thrombolysis.

HYPEROXIA IN GLOBAL CEREBRAL ISCHEMIA

Global cerebral ischemia invariably complicates conditions such as cardiac arrest, shock and respiratory arrest. Despite promising pre-clinical results, no pharmacologic intervention after cardiac arrest has been successfully translated to human neuroprotection. The only strategy that has proved successful is induced hypothermia, which targets multiple cell death pathways. Hyperoxia, with its pluripotent effects on excitotoxicity, apoptosis and inflammation, appears to be a promising alternative strategy.

Several studies have shown benefit with HBO after global cerebral ischemia. Rosenthal et al. showed that 1 hour HBO administration after 10 minute cardiac arrest in dogs reduces neuronal loss and neurological outcome. In another canine study, repeated sessions of HBO therapy after 15 minute cardiac arrest significantly improved neurological and electrophysiological outcomes and survival rates as compared to room air-treated control animals. In a rodent four-vessel occlusion model, the 14 day survival rate was 45% with HBO and 0% with room air, and in a rodent two-vessel occlusion model, 2 hour HBO therapy starting 1 hour after global ischemia significantly reduced neurological injury. The results of a recent animal study suggest that HBO may also be a promising therapeutic strategy in neonatal hypoxic–ischemic encephalopathy. In this study, HBO at 3 ATA applied for 1 hour after cerebral hypoxia–ischemia in 7 days old rat pups resulted in a reduction in brain atrophy and tissue loss at weeks 2 and 6 as assessed by light and electron microscopy. Furthermore, HBO improved sensorimotor function assessed by the postural reflex test.

Other studies have shown that hyperoxia can be detrimental after cardiac arrest. Gerbils treated with 100% oxygen for 3–6 hours after 15 minute bilateral carotid occlusion developed sustained white matter damage although the neurons appeared better preserved, and developed increased lipid peroxidation and a three-fold increase in 14 day mortality as compared to animals treated with room air. In a canine model of 10 minute cardiac arrest, Fiskum’s group found 40% reduction in hippocampal neuronal death, lower levels of oxidized brain lipids and improved neurological outcome using normoxic as compared with hyperoxic resuscitation. Zwemer et al. have similarly shown in canine models that normoxia rather than hyperoxia or hypoxia is the ideal target for arterial oxygenation during resuscitation. While these adverse effects have been attributed to increased free radical generation during hyperoxic resuscitation, the findings of at least three studies suggest that hyperoxia-induced free radical generation does not influence final brain damage or neurological outcome.

In light of these conflicting data, it is important to consider the influence of timing on the efficacy of hyperoxia after cardiac arrest. In one study, as compared to rats treated with HBO starting 1 hour after global ischemia, rats treated with HBO at hour 3 had higher ATP levels, lower lactate levels and better survival. It is conceivable that neurological outcome after cardiac arrest can be optimized by maintaining normoxia during the early reperfusion period when there is massive generation of toxic oxygen free radicals and hyperoxia during later periods when there is a rise in cerebral energy metabolism and a decline in cerebral blood flow. Under current protocols, oxygen is freely administered to all patients with cardiac arrest. Further studies are warranted to determine the optimal levels of post-ischemic oxygenation at serial time points after cardiac arrest.

SAFETY CONCERNS

The major safety concern with oxygen therapy is increased generation of toxic free radicals, which in the setting of ischemic stroke could exacerbate brain edema, post-ischemic hemorrhage and tissue necrosis. Few studies have examined whether the risk/benefit ratio of hyperoxia in ischemic stroke changes with the timing or the duration of therapy, and whether hyperoxia increases free radical generation in the brain to levels of clinically meaningful toxicity. Using a rabbit model global ischemia–resperfusion, Mink and Dutka showed that HBO exposure for 75 minutes at 2.8 ATA did increase free radical generation; however, this did not translate to increased lipid peroxidation, and HBO-treated animals had better neurophysiologic outcomes. In at least two studies of transient cerebral ischemia, hyperoxia did not increase free radical generation. The risks of hyperoxia have mainly been demonstrated with prolonged (greater than 24–48 hours) exposure in neonates and in animal models of post-ischemic hypoxia such as global cerebral ischemia–reperfusion. As discussed, oxygen’s effects appear to be beneficial in

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clinically relevant models such as transient focal cerebral ischemia where collateral circulation is preserved and therapy is brief.

Oxygen inhalation can increase shunt fraction and decrease lung volumes due to absorptive atelectasis, and accentuate hypercapnia and suppress the hypoxic respiratory drive in patients with active chronic obstructive pulmonary disease, resulting in possible intubation.

Hyperoxia can also lead to systemic vasoconstriction, reduced cardiac output and bradycardia. However, these effects are mild, fully reversible and their clinical significance is unclear. Hyperoxia is not known to cause systemic hypotension which could potentially exacerbate cerebral ischemia. Another concern is hyperoxia-induced cerebral vasodilation, which could theoretically compromise blood flow to ischemic brain. As discussed above, oxygen-induced vasodilation occurs in the normal brain and not in the ischemic brain where blood flow paradoxically increases. HBO might support ischemic tissues by inducing an 'inverse steal' effect, and the hemodynamic changes observed in recent NBO studies support this possibility.

The use of hyperoxia in the neonatal period is controversial. Although international guidelines recommend the use of 100% oxygen for the resuscitation of newborns, hyperoxia-induced free radical generation is believed to contribute to neonatal complications such as retinopathy of prematurity and lung injury. A meta-analysis of published studies showed that newborns tend to have lower mortality rates when resuscitated with room air as opposed to 100% oxygen. Nevertheless, it is important to understand that the risks of hyperoxia are generally associated with prolonged exposure and depend on factors such as stage of tissue maturity and the dose of hyperoxia. For example, Dombińska et al. have shown that the risk for retinopathy of prematurity increases with prolonged durations of hyperoxia, and is higher in the second week of life as compared with the first, third or fourth weeks. As with ischemic stroke, early timing and short durations of hyperoxia may in fact be beneficial. Zhang and colleagues recently showed that a single 1 hour exposure to 100% oxygen at normobaric and hyperbaric pressures (1, 1.5 and 3.0 ATA), does not promote retinopathy of prematurity in 7 day rat pups. None of the three doses of hyperoxia caused thinning of the outer plexiform layer or abnormal retinal vascularization, which are the structural hallmarks of retinopathy of prematurity. Further studies are warranted to investigate the safety and therapeutic potential of HBO in neonatal hypoxic–ischemic encephalopathy.

HBO has been shown to promote free radical-mediated tissue injury when used in conjunction with disulfiram, Adriamycin, bleomycin and other chemotherapeutic agents. Adverse effects more specific to HBO include pulmonary and aural barotrauma, seizures, claustrophobia, injuries related to chamber fires and reduced visual acuity from conformational changes in the lens. None of the three clinical trials, however, reported any serious adverse events and HBO is generally considered a benign therapy.

**HBO IN OTHER CEREBROVASCULAR DISEASES**

HBO is known to reduce cerebral edema due to its vasoconstrictive and metabolic effects, and by preserving BBB integrity. Mink and Dutka found that HBO was more effective than NBO in reducing vascular permeability. Similarly, Veltkamp et al. recently showed that HBO was superior to NBO and room air, in reducing the volume of infarct on serial MRI and the extent of BBB damage at early and delayed time points after experimental focal stroke. In their study, BBB damage was assessed by comparing post-contrast enhancement on T1-weighted images and Na-fluorescein tracer extravasation, in treated versus control animals. Sukhoff et al. have shown that HBO reduces intracranial pressure and brain lactate levels in animal models and humans with traumatic brain injury. Continuing studies of traumatic brain injury by Ross Bullock's group further support the efficacy of hyperoxia in treating brain edema. HBO may be effective in treating spinal cord infarction and spinal trauma. Zhang's group showed that a single session of HBO within 3 hours or multiple HBO treatments within 24 hours, improves histopathologic and functional outcomes after spinal cord injury in rats. Anecdotal reports suggest that HBO may be effective in treating primary brain hemorrhage, and some authors have even suggested that clinical improvement during HBO exposure could be used to select patients appropriate for surgical hematoma evacuation. Finally, animal studies as well as human studies have demonstrated the use of HBO in treating vasospasm-induced cerebral ischemia after subarachnoid hemorrhage.

**NBO IN FOCAL ISCHEMIC STROKE**

Based on the above discussion, it appears that the failure of the previous HBO stroke trials is not attributable to the lack of efficacy of oxygen, but rather to shortcomings in clinical trial design. Theoretically, any method of supplemental oxygenation would prove efficacious, provided factors such as the timing of therapy, dose of oxygen and the presence of salvageable tissue are taken into consideration. Several groups have become interested in NBO because it offers distinct advantages over HBO: it is widely available, simple to administer, inexpensive and most importantly, can be started very quickly after stroke onset, e.g. by paramedics.

The results of recent rodent studies, all using the filament model of transient middle cerebral artery ischemia-reperfusion, show that NBO improves pathologic, neurobehavioural and neuroimaging outcomes after stroke. We have shown that NBO therapy administered during ischemia and in the immediate post-reperfusion period results in a 70% reduction in hemispheric infarct volumes. The benefit is most pronounced in the cerebral cortex, where infarct...
volumes are reduced by as much as 90% as compared to normoxic controls. The therapeutic time window for NBO in rodents is short (approximately 30–45 minutes); initiating treatment at earlier time points enhances the degree of neuroprotection. Furthermore, NBO therapy attenuates the severity and the volume of ischemic injury assessed by serial DWI. Finally, we have shown that NBO therapy administered shortly after stroke onset can extend the ‘reperfusion time window’ from 1 to 3 hours. In similar experiments, Flynn and Auer have shown that NBO therapy reduces functional deficits, brain atrophy and weight loss after stroke in rodents. Using in vivo EPR oximetry, Liu’s group has shown that NBO significantly increases brain interstitial pO2 in penumbral tissues, suggesting that NBO can be used to salvage ischemic brain.

At least two studies have addressed the presumption that NBO is ‘ineffective’ in raising brain tissue oxygen levels. Flynn and Auer showed that pO2 values above 200 mm Hg, which are easily achieved with NBO, do not confer additional benefit. Liu’s group has recently shown that 95% oxygen administered during ischemia can maintain penumbral interstitial pO2 levels close to the pre-ischemic values. While the increase in brain ptiO2 affected by NBO is certainly minor as compared to HBO, the critical oxygen tension required for mitochondrial function is extremely low and even small increases in ptiO2 might be adequate to overcome thresholds for ischemic cell death.

The theoretical concern of exacerbating oxygen free radical-associated reperfusion injury with NBO has also been investigated. Flynn and Auer showed that NBO administered solely during the post-reperfusion phase reduces infarct volumes, which is highly relevant given our knowledge that free radicals are mainly generated during tissue reperfusion. We have shown that NBO does not increase hydroethidine (a cellular marker of oxidative stress such as matrix metalloproteinase 2 (MMP-2), MMP-9, heat shock protein 32 and protein carbonyl formation, at acute or subacute time points after stroke. Liu’s group has similarly shown that NBO therapy in focal stroke results in lower levels of 8-OhdG and hydroethidine (both measures of reactive oxygen species), MMP-9 and caspase-8.

The effects of oxygen inhalation in stroke have been evaluated in two human studies. The first was an observational study from Norway, where 1-year survival was better in room air-treated control patients with mild–moderate stroke as compared to those treated with 3 l/min nasal oxygen. The authors concluded that non-hypoxic stroke victims with mild–moderate strokes should not receive supplemental oxygen. However, this was an observational study where patients received very low doses (3 l/min) of oxygen, for as long as 24 hours, and the time to treatment was relatively late (~40% of patients had unknown time of onset or were treated after 12–24 hours). Moreover, 18% patients did not receive the assigned ‘therapy’, 12.7% had hemorrhagic stroke and no attention was paid to stroke pathophysiology. Finally, outcome (survival) was measured after 1 year. It is difficult to ascertain the true safety or efficacy of oxygen in ischemic stroke patients on the basis of this study.

Our group recently published results of the first therapeutic trial of NBO (45 l/min oxygen administered via a facemask). In this study, 16 patients with hemispheric ischemic stroke symptoms less than 12 hours and DWI–PWI mismatch on admission MRI were randomized to 8 hours of NBO or room air. DWI and PWI were performed before treatment (baseline), during treatment, after treatment was stopped, and at week 1 and month 3. NBO-treated patients showed improvement in NIHSS scores, reduced growth of DWI lesion volumes and an increase in the volume of ‘penumbral’ tissue while therapy was being...
administered (Figure 4). Remarkably, NBO therapy resulted in improvement of visible DWI lesions, which has previously been documented only with prompt arterial recanalization11,12. In addition to the manual volumetric MRI analysis, we performed automated voxel-by-voxel analysis to determine the change in the intensity of ADC voxels from the baseline MRI to ‘during therapy’ and ‘post-therapy’ MRI scans. The percentage of MRI voxels improving from baseline ‘ischemic’ to 4 hour ‘non-ischemic’ values tended to be higher in hyperoxia-treated patients. In a subset of patients studied with serial MR spectroscopy, NBO therapy was found to improve brain lactate levels within regions of ischemia21. There was no clinical or radiologic evidence of oxygen toxicity in this study. In light of these data, it can be hypothesized that stroke patients would benefit from receiving NBO in the field, followed by HBO and if eligible, tPA upon arrival to the hospital.

SUMMARY AND FUTURE DIRECTIONS
Neuroprotective drugs have so far failed clinical trials, at high cost113,114, and i.v. tPA remains the only FDA-approved acute stroke treatment. The available animal and human data suggest that hyperoxia, acting via multiple direct and indirect mechanisms, may be a powerful neuroprotective strategy that can salvage ischemic brain tissue, extend the time window for acute stroke treatment, enhance post-stroke recovery and perhaps pre-condition against subsequent stroke. Of the available oxygen delivery methods, HBO is probably the most potent. However, even NBO may be effective if applied early enough after stroke onset. There is growing recognition that the failure of previous HBO clinical trials may have resulted from factors such as delayed time to therapy, inadequate sample size and use of excessive chamber pressures. Past trials did not assess long-term benefit in patients with tissue reperfusion, and subsequent tissue reperfusion may be a requisite for long-term benefit. Today, the advent of stroke thrombolysis with higher reperfusion rates offers the unique opportunity to investigate the efficacy of a combined hyperoxia/reperfusion strategy. Major challenges to developing HBO as an acute stroke therapy include patient non-compliance, limited availability of HBO chambers, minor commercial potential and difficulties in delivering medical care to acutely ill patients within chambers particularly in this era of stroke thrombolysis. Conceivably, a widely available therapy such as NBO could be initiated in the field, followed by HBO or if appropriate, thrombolytic therapy after hospital arrival. In turn, hyperoxia (NBO or HBO) may be a useful strategy to extend the thrombolysis time window, which would significantly increase the use of tPA in regions around the world where immediate access to acute stroke care is limited.

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