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 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[1]. 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[2], 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[2–4]. Until this time, hyperbaric air and not oxygen was used. In 1960, Boerema published a landmark study showing

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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[5]. 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[4]. Ingvar and Lassen from Lund University published a report on the use of HBO in four patients with stroke[6]. 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[7]. 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[8,9]. 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 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[4]. 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[10]. This study is known to have several shortcomings[11–13] 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[14]. In humans, the results of position 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 pO_2 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 NBO 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[15]. Tissue

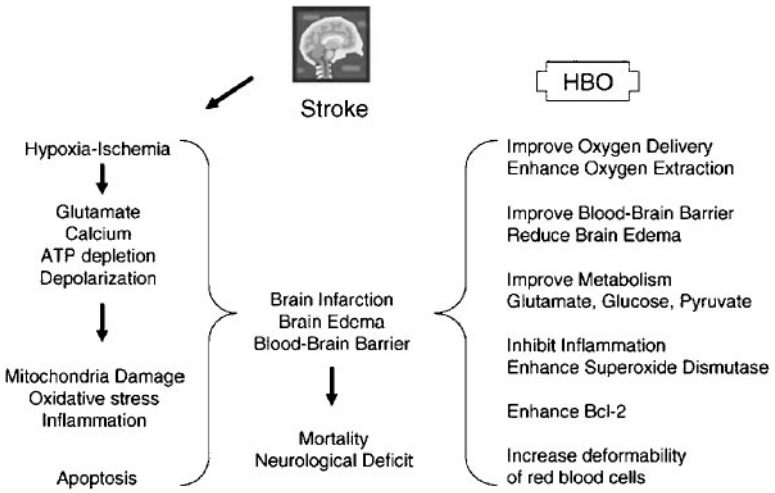

Figure 1: Mechanisms of HBO neuroprotection. Cerebral hypoxia–ischemia disables energy metabolism, reduces ATP production, releases glutamate, and causes calcium overload and depolarization. Mitochondrial damage follows, with oxygen radical generation and inflammatory reactions. All these pathologic 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 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[42]

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[16]. In models of traumatic brain injury, HBO increased brain tissue pO_2, increased the cerebral metabolic rate of oxygen, decreased brain lactate and pyruvate levels, and improved mitochondrial function[17,18]. Recent studies indicate that NBO has similar effects. Electron paramagnetic resonance (EPR) oximetry studies have shown that NBO improves penumbral oxygenation after rodent stroke[19,20]. Preliminary studies using serial MR spectroscopy suggest that NBO improves lactate levels within ischemic regions in humans with ischemic stroke[21]. In patients with brain trauma, NBO improved brain lactate and pyruvate levels and decreased intracranial pressure[22]. Diffusion-weight MRI (DWI) abnormalities after ischemic stroke result from ion pump failure, and reversal of such abnormalities with HBO and NBO therapy indicates that hyperoxia restores ion pump function[23–25]. Finally, by increasing oxygen levels, HBO might inhibit post-anoxic depolarization[26] 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[27] and decreased polymorphonuclear cell infiltration[28]. 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[29]. 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[30], neonatal hypoxic–ischemic brain injury[31] and brain trauma[32]. 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[33]. However, it is important to understand that local pO_2 increases with HBO therapy despite its cerebral blood flow lowering effects[33], 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[34,35]). That NBO may have similar hemodynamic effects is suggested by serial perfusion-MRI findings of recent rodent and human stroke studies[24,25]. 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[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[37], and inhibit the Nogo-A pathways[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[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 *et al.*[42] and Helms *et al.*[43] have recently summarized these results in excellent review articles. HBO has been studied in models of transient focal stroke[23,27,28,30,44–52] as well as permanent focal stroke[16,51,53–57]. The rodent model has been used in most of these studies, with gerbils[53–55] and cats[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.[43] In general, single sessions have been used, with only few studies investigating the effects of multiple sessions[44,47,58]. Positive results have been documented in all but three studies[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[16,51,53–56]. At least three studies[46,51,59] have shown that the efficacy of HBO diminishes with longer period of ischemia. In a study by Veltkamp *et al.*[49], 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[12].

Several studies have investigated the therapeutic time window of HBO. Weinstein *et al.* administered HBO at 1.5 ATA for 40 minutes during and after 6 hour MCAO, and before, during and after 24 hour MCAO in unanesthetized cats[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[59] (*Figure 2*). Similarly, Lou *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[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[58]. Supporting the efficacy of multiple treatments is the fact that such regimens also extend the therapeutic time window for acute spinal cord injury[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 *et al.*[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 *et al.*[23] performed serial MRI at minute 15 and hours 3, 6 and 24 in Wistar rats treated

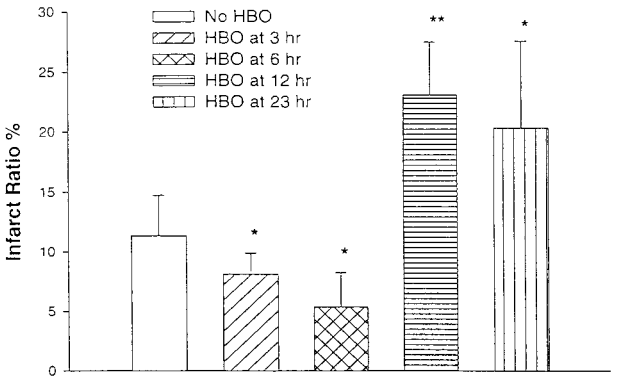

Figure 2: Time window of HBO in rodents. Rats were subjected to 2 hour MCAO and treated with HBO (3 ATA for 1 hour) 3, 6, 12 or 24 hours post-reperfusion. Infarct ratio was calculated as the percentage of infarcted tissue per ipsilateral hemisphere. In the control group (non-HBO therapy), the infarct ratio is ~11.34%. HBO treatment at hours 3 and 6 markedly reduced the infarct ratio, whereas HBO treatment at hours 12 and 23 increased the infarct ratio (*$p<0.05$ and **$p<0.01$ compared with the control group, ANOVA). These findings suggest that the time window for HBO after transient focal stroke in rodents is 6 hours[59]

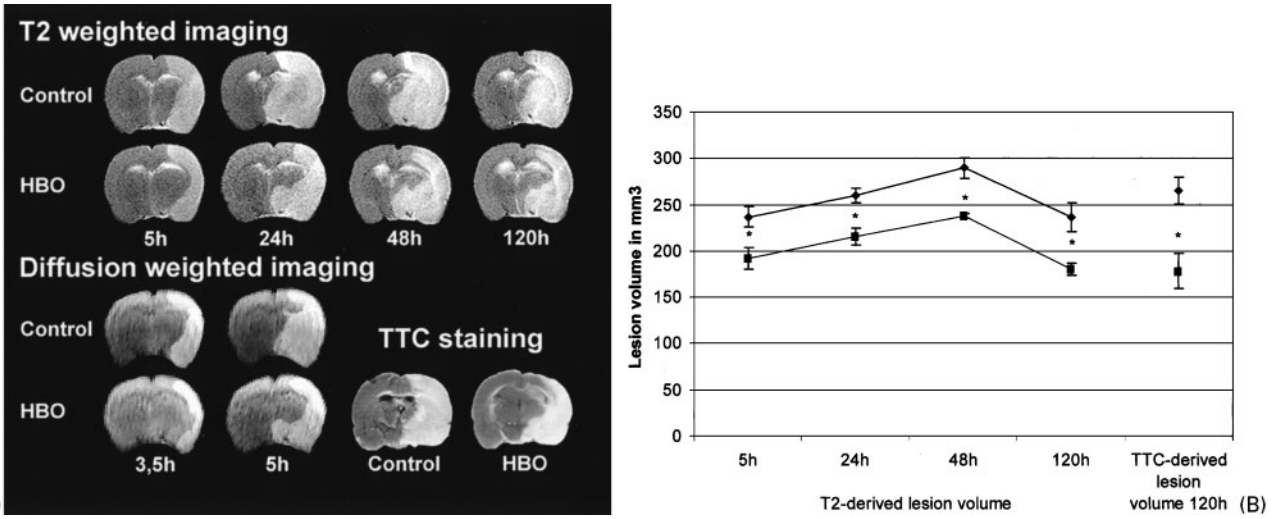

Figure 3: MRI monitoring of HBO's effects in focal stroke. (**A**) Ischemic lesion evolution measured by DWI and T2-weighted imaging in rodents treated with room air or HBO after permanent MCAO. Post-mortem triphenyltetrazolium chloride (TTC) staining on day 5 is shown on the lower right. Note the long-lasting (5 day) neuroprotection achieved with HBO, effective as early as 5 hours after vessel occlusion. (**B**) Serial changes in T2-weighted ischemic lesion volumes in HBO-treated animals (squares) and controls (diamonds) after permanent focal cerebral ischemia. Neuroprotection began 5 hours after ischemia, remained effective throughout 5 days and correlated with post-mortem TTC-derived infarct volume. Values are expressed as mean ± SD (*$p<0.05$; ANOVA, Fisher's test); time is given non-linear in days (x axis)[56]

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[49]. 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[2,61–68]. 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[62,63], improve recovery after stroke[66,67] and predict success from cerebral revascularization procedures[68]. 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[8–10]. Anderson *et al.*[8] 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.*[9] 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.*[10] 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[69]. 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.*[8], 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.*[10]. 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[12]; 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[11]. 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[70,71] 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.*[72] 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[73]. In a rodent four-vessel occlusion model, the 14 day survival rate was 45% with HBO and 0% with room air[74], and in a rodent two-vessel occlusion model, 2 hour HBO therapy starting 1 hour after global ischemia significantly reduced neurological injury[38]. The results of a recent animal study suggest that HBO may also be a promising therapeutic strategy in neonatal hypoxic–ischemic encephalopathy[31]. 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[75] 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[76]. 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[77,78]. 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[79,80]. 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.[81–83] 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[84]. 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[85] 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[86,87], 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-reperfusion, Mink and Dutka[82] 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[16,81]. 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 hyperoxia such as global cerebral ischemia-reperfusion[76,78]. As discussed, oxygen's effects appear to be beneficial in

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[88]. 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[89,90]. Hyperoxia is not known to cause systemic hypotension which could potentially exacerbate cerebral ischemia. Another concern is hyperoxia-induced cerebral vasoconstriction[91], which could theoretically compromise blood flow to ischemic brain. As discussed above, oxygen-induced vasoconstriction occurs in the normal brain and not in the ischemic brain where blood flow paradoxically increases[92]. HBO might support ischemic tissues by inducing an 'inverse steal'[34,35] effect, and the hemodynamic changes observed in recent NBO studies[24,25] 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[93]. A meta-analysis of published studies showed that neonates tend to have lower mortality rates when resuscitated with room air as opposed to 100% oxygen[94]. 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, Dembinska *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[95]. 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[96]. 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[97], adriamycin[98], bleomycin[99] 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[100] found that HBO was more effective than NBO in reducing vascular permeability. Similarly, Veltkamp *et al.*[36] 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[36]. Sukhoff *et al.*[101,102] 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[17,22]. HBO may be effective in treating spinal cord infarction[103] and spinal trauma[60]. 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[60]. Anecdotal reports suggest that HBO may be effective in treating primary brain hemorrhage[104], and some authors have even suggested that clinical improvement during HBO exposure could be used to select patients appropriate for surgical hematoma evacuation[105]. Finally, animal[106] as well as human[105] 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[24,107]. The benefit is most pronounced in the cerebral cortex, where infarct

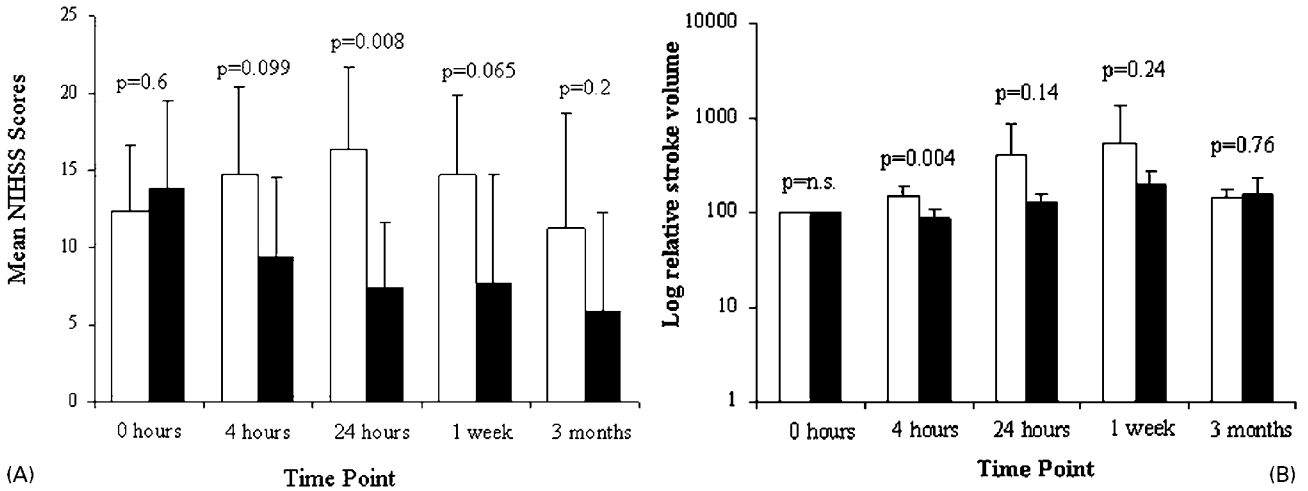

Figure 4: Therapeutic efficacy of NBO in patients with acute ischemic stroke. Patients with acute ischemic stroke <12 hours were randomized to room air (controls) or NBO (45 l/min oxygen) for 8 hours. NIHSS scores (**A**) and relative MRI stroke lesion volumes (**B**) were similar at baseline, improved significantly in the NBO-treated group after therapy was initiated, but were not significantly different at chronic time points[25]

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[24]. Furthermore, NBO therapy attenuates the severity and the volume of ischemic injury assessed by serial DWI[24]. Finally, we have shown that NBO therapy administered shortly after stroke onset can extend the 'reperfusion time window' from 1 to 3 hours[108]. In similar experiments, Flynn and Auer[109] 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 pO_2 in penumbral tissues[19], 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[109] showed that pO_2 values above 200 mm Hg, which are easily achieved with NBO, do not confer additional benefit. Liu's group[20] has recently shown that 95% oxygen administered during ischemia can maintain penumbral interstitial pO_2 levels close to the pre-ischemic values. While the increase in brain $ptiO_2$ affected by NBO is certainly minor as compared to HBO, the critical oxygen tension required for mitochondrial function is extremely low[110] and even small increases in $ptiO_2$ 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[109] 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 superoxide generation), does not significantly worsen BBB damage and does not increase the levels of indirect markers 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[107,108]. Liu's group[20] 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[111] where outcome (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)[25]. 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 recanalization[112]. 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 ischemia[21]. 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 cost[113,114], 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.

REFERENCES

1 Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rT-PA Stroke Study Group. *N Engl J Med* 1995; **333**: 1581–1587
2 Jain KK. *Textbook of Hyperbaric Medicine*, Gottingen: Hogrefe & Huber, 2004
3 Jain KK. Hyperbaric oxygenation for the treatment of stroke. In: Gilman S, ed. *Medlink*. Available at: http://www.medlink.com[2004]
4 Jain KK, Toole JF, eds. *Hyperacute Hyperbaric Oxygen Therapy for Stroke: Proceedings of A Conference*, Bethesda, ML: Undersea and Hyperbaric Medical Society, 1998
5 Jacobson I, Lawson DD. Effect of hyperbaric oxygen on experimental cerebral infarction in dog with preliminary correlations of cerebral blood flow at 2 atmospheres of oxygen. *J Neurosurg* 1963; **20**: 849–859
6 Ingvar HD, Lassen NA. Treatment of focal cerebral ischemia with hyperbaric oxygen. *Acta Neurol Scand* 1965; **41**: 92–95
7 Neubauer RA, End E. Hyperbaric oxygenation as an adjunct therapy in strokes due to thrombosis. A review of 122 patients. *Stroke* 1980; **11**: 297–300
8 Anderson DC, Bottini AG, Jagiella WM, *et al.* A pilot study of hyperbaric oxygen in the treatment of human stroke. *Stroke* 1991; **22**: 1137–1142
9 Nighoghossian N, Trouillas P, Adeleine P, *et al.* Hyperbaric oxygen in the treatment of acute ischemic stroke. A double-blind pilot study. *Stroke* 1995; **26**: 1369–1372
10 Rusyniak DE, Kirk MA, May JD, *et al.* Hyperbaric oxygen therapy in acute ischemic stroke: Results of the hyperbaric oxygen in acute ischemic stroke trial pilot study. *Stroke* 2003; **34**: 571–574
11 Zhang JH, Singhal AB, Toole JF. Oxygen therapy in ischemic stroke. *Stroke* 2003; **34**: e152–e153
12 Jain KK. Hyperbaric oxygen in acute ischemic stroke. *Stroke* 2003; **34**: e153
13 Hart GB, Strauss MB. Hyperbaric oxygen therapy. *Stroke* 2003; **34**: e153–e155
14 Baron JC. Perfusion thresholds in human cerebral ischemia: Historical perspective and therapeutic implications. *Cerebrovasc Dis* 2001; **11** (Suppl. 1): 2–8
15 Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. *Nat Rev Neurosci* 2003; **4**: 399–415
16 Sunami K, Takeda Y, Hashimoto M, *et al.* Hyperbaric oxygen reduces infarct volume in rats by increasing oxygen supply to the ischemic periphery. *Crit Care Med* 2000; **28**: 2831–2836
17 Daugherty WP, Levasseur JE, Sun D, *et al.* Effects of hyperbaric oxygen therapy on cerebral oxygenation and mitochondrial function following moderate lateral fluid-percussion injury in rats. *J Neurosurg* 2004; **101**: 499–504
18 Rockswold SB, Rockswold GL, Vargo JM, *et al.* Effects of hyperbaric oxygenation therapy on cerebral metabolism and intracranial pressure in severely brain injured patients. *J Neurosurg* 2001; **94**: 403–411
19 Liu S, Shi H, Liu W, *et al.* Interstitial pO_2 in ischemic penumbra and core are differentially affected following transient focal cerebral ischemia in rats. *J Cereb Blood Flow Metab* 2004; **24**: 343–349
20 Liu S, Liu W, Ding W, *et al.* EPR-guided normobaric hyperoxia treatment protects the brain by maintaining penumbral oxygenation in a rat model of transient focal cerebral ischemia. *J Cereb Blood Flow Metab* 2006; **26(10)**: 1274–1284
21 Ratai E, Benner T, Sorensen A, *et al.* Therapeutic effects of normobaric hyperoxia in acute stroke: Multivoxel MR-spectroscopy and ADC correlations on serial imaging. Present at *International Society of Magnetic Resonance Imaging in Medicine*, 2005, Miami, FL, USA
22 Tolias CM, Reinert M, Seiler R, *et al.* Normobaric hyperoxia-induced improvement in cerebral metabolism and reduction in intracranial pressure in patients with severe head injury: A prospective historical cohort-matched study. *J Neurosurg* 2004; **101**: 435–444
23 Veltkamp R, Siebing DA, Heiland S, *et al.* Hyperbaric oxygen induces rapid protection against focal cerebral ischemia. *Brain Res* 2005; **1037**: 134–138
24 Singhal AB, Dijkhuizen RM, Rosen BR, *et al.* Normobaric hyperoxia reduces MRI diffusion abnormalities and infarct size in experimental stroke. *Neurology* 2002; **58**: 945–952
25 Singhal AB, Benner T, Roccatagliata L, *et al.* A pilot study of normobaric oxygen therapy in acute ischemic stroke. *Stroke* 2005; **36**: 797–802

26 Hossmann KA. Viability thresholds and the penumbra of focal ischemia. *Ann Neurol* 1994; **36**: 557–565
27 Yin W, Badr AE, Mychaskiw G, *et al.* Down regulation of COX-2 is involved in hyperbaric oxygen treatment in a rat transient focal cerebral ischemia model. *Brain Res* 2002; **926**: 165–171
28 Miljkovic-Lolic M, Silbergleit R, Fiskum G, *et al.* Neuroprotective effects of hyperbaric oxygen treatment in experimental focal cerebral ischemia are associated with reduced brain leukocyte myeloperoxidase activity. *Brain Res* 2003; **971**: 90–94
29 Buras JA, Stahl GL, Svoboda KK, *et al.* Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: The role of NOS. *Am J Physiol Cell Physiol* 2000; **278**: C292–C302
30 Yin D, Zhou C, Kusaka I, *et al.* Inhibition of apoptosis by hyperbaric oxygen in a rat focal cerebral ischemic model. *J Cereb Blood Flow Metab* 2003; **23**: 855–864
31 Calvert JW, Yin W, Patel M, *et al.* Hyperbaric oxygenation prevented brain injury induced by hypoxia–ischemia in a neonatal rat model. *Brain Res* 2002; **951**: 1–8
32 Vlodavsky E, Palzur E, Feinsod M, *et al.* Evaluation of the apoptosis-related proteins of the bcl-2 family in the traumatic penumbra area of the rat model of cerebral contusion, treated by hyperbaric oxygen therapy: A quantitative immunohistochemical study. *Acta Neuropathol (Berl)* 2005; **110**: 120–126
33 Demchenko IT, Boso AE, Bennett PB, *et al.* Hyperbaric oxygen reduces cerebral blood flow by inactivating nitric oxide. *Nitric Oxide* 2000; **4**: 597–608
34 Lassen NA, Palvögyi R. Cerebral steal during hypercapnia and the inverse reaction during hypocapnia observed by the 133-xenon technique in man. *Scand J Clin Lab Invest* 1968; **22** (Suppl. 102): XIIID
35 Nighoghossian N, Trouillas P. Hyperbaric oxygen in the treatment of acute ischemic stroke: An unsettled issue. *J Neurol Sci* 1997; **150**: 27–31
36 Veltkamp R, Siebing DA, Sun L, *et al.* Hyperbaric oxygen reduces blood–brain barrier damage and edema after transient focal cerebral ischemia. *Stroke* 2005; **36**: 1679–1683
37 Conconi MT, Baiguera S, Guidolin D, *et al.* Effects of hyperbaric oxygen on proliferative and apoptotic activities and reactive oxygen species generation in mouse fibroblast 3t3/j2 cell line. *J Investig Med* 2003; **51**: 227–232
38 Zhou C, Li Y, Nanda A, *et al.* HBO suppresses NOGO-a, NG-r, or RHOa expression in the cerebral cortex after global ischemia. *Biochem Biophys Res Commun* 2003; **309**: 368–376
39 Xiong L, Zhu Z, Dong H, *et al.* Hyperbaric oxygen preconditioning induces neuroprotection against ischemia in transient not permanent middle cerebral artery occlusion rat model. *Chin Med J (Engl)* 2000; **113**: 836–839
40 Prass K, Wiegand F, Schumann P, *et al.* Hyperbaric oxygenation induced tolerance against focal cerebral ischemia in mice is strain dependent. *Brain Res* 2000; **871**: 146–150
41 Dong H, Xiong L, Zhu Z, *et al.* Preconditioning with hyperbaric oxygen and hyperoxia induces tolerance against spinal cord ischemia in rabbits. *Anesthesiology* 2002; **96**: 907–912
42 Zhang JH, Lo T, Mychaskiw G, *et al.* Mechanisms of hyperbaric oxygen and neuroprotection in stroke. *Pathophysiology* 2005; **12**: 63–77
43 Helms AK, Whelan HT, Torbey MT. Hyperbaric oxygen therapy of cerebral ischemia. *Cerebrovasc Dis* 2005; **20**: 417–426
44 Chang CF, Niu KC, Hoffer BJ, *et al.* Hyperbaric oxygen therapy for treatment of postischemic stroke in adult rats. *Exp Neurol* 2000; **166**: 298–306
45 Yang ZJ, Camporesi C, Yang X, *et al.* Hyperbaric oxygenation mitigates focal cerebral injury and reduces striatal dopamine release in a rat model of transient middle cerebral artery occlusion. *Eur J Appl Physiol* 2002; **87**: 101–107
46 Weinstein PR, Anderson GG, Telles DA. Results of hyperbaric oxygen therapy during temporary middle cerebral artery occlusion in unanesthetized cats. *Neurosurgery* 1987; **20**: 518–524
47 Roos JA, Jackson-Friedman C, Lyden P. Effects of hyperbaric oxygen on neurologic outcome for cerebral ischemia in rats. *Acad Emerg Med* 1998; **5**: 18–24
48 Atochin DN, Fisher D, Demchenko IT, *et al.* Neutrophil sequestration and the effect of hyperbaric oxygen in a rat model of temporary middle cerebral artery occlusion. *Undersea Hyperb Med* 2000; **27**: 185–190
49 Veltkamp R, Warner DS, Domoki F, *et al.* Hyperbaric oxygen decreases infarct size and behavioral deficit after transient focal cerebral ischemia in rats. *Brain Res* 2000; **853**: 68–73
50 Badr AE, Yin W, Mychaskiw G, *et al.* Effect of hyperbaric oxygen on striatal metabolites: A microdialysis study in awake freely moving rats after MCA occlusion. *Brain Res* 2001; **916**: 85–90
51 Lou M, Eschenfelder CC, Herdegen T, *et al.* Therapeutic window for use of hyperbaric oxygenation in focal transient ischemia in rats. *Stroke* 2004; **35**: 578–583
52 Kawamura S, Yasui N, Shirasawa M, *et al.* Therapeutic effects of hyperbaric oxygenation on acute focal cerebral ischemia in rats. *Surg Neurol* 1990; **34**: 101–106
53 Corkill G, Van Housen K, Hein L, *et al.* Videodensitometric estimation of the protective effect of hyperbaric oxygen in the ischemic gerbil brain. *Surg Neurol* 1985; **24**: 206–210
54 Burt JT, Kapp JP, Smith RR. Hyperbaric oxygen and cerebral infarction in the gerbil. *Surg Neurol* 1987; **28**: 265–268
55 Reitan JA, Kien ND, Thorup S, *et al.* Hyperbaric oxygen increases survival following carotid ligation in gerbils. *Stroke* 1990; **21**: 119–123
56 Schabitz WR, Schade H, Heiland S, *et al.* Neuroprotection by hyperbaric oxygenation after experimental focal cerebral ischemia monitored by MRI. *Stroke* 2004; **35**: 1175–1179
57 Hjelde A, Hjelstuen M, Haraldseth O, *et al.* Hyperbaric oxygen and neutrophil accumulation/tissue damage during permanent focal cerebral ischaemia in rats. *Eur J Appl Physiol* 2002; **86**: 401–405
58 Yin D, Zhang JH. Delayed and multiple hyperbaric oxygen treatments expand therapeutic window in rat focal cerebral ischemic model. *Neurocrit Care* 2005; **2**: 206–211
59 Badr AE, Yin W, Mychaskiw G, *et al.* Dual effect of HBO on cerebral infarction in MCAO rats. *Am J Physiol Regul Integr Comp Physiol* 2001; **280**: R766–R770
60 Huang L, Mehta MP, Nanda A, *et al.* The role of multiple hyperbaric oxygenation in expanding therapeutic windows after acute spinal cord injury in rats. *J Neurosurg* 2003; **99**: 198–205
61 Sarno JE, Rusk HA, Diller L, *et al.* The effect of hyperbaric oxygen on the mental and verbal ability of stroke patients. *Stroke* 1972; **3**: 10–15
62 Lebedev VV, Isakov Iu V, Pravdenkova SV. [Effect of hyperbaric oxygenation on the clinical course and complications of the acute period of ischemic strokes]. *Zh Vopr Neirokhir Im N N Burdenko* 1983; (3): 37–42
63 Pravdenkova SV, Romasenko MV, Shelkovskii VN. [Hyperbaric oxygenation and prevention of recurrent cerebral circulatory disorders in the acute stage of a stroke]. *Zh Nevropatol Psikhiatr Im S S Korsakova* 1984; **84**: 1147–1151
64 Kaasik AE, Dmitriev KK, Tomberg TA. [Hyperbaric oxygenation in the treatment of patients with ischemic stroke]. *Zh Nevropatol Psikhiatr Im S S Korsakova* 1988; **88**: 38–43
65 Neubauer RA. Generalized small-vessel stenosis in the brain. A case history of a patient treated with monoplace hyperbaric oxygen at 1.5 to 2 ATA. *Minerva Med* 1983; **74**: 2051–2055
66 Holbach KH, Wassmann HW, Hoheluchter KL. Reversibility of the chronic post-stroke state. *Stroke* 1976; **7**: 296–300
67 Wassmann H. [Stroke therapy. Combined therapy of hyperbaric oxygenation and extra- intracranial arterial bypass operation]. *Fortschr Med* 1982; **100**: 285–288
68 Rossi GF, Maira G, Vignati A, *et al.* Neurological improvement in chronic ischemic stroke following surgical brain revascularization. *Ital J Neurol Sci* 1987; **8**: 465–475
69 Bennett MH, Wasiak J, Schnabel A, *et al.* Hyperbaric oxygen therapy for acute ischaemic stroke. *Cochrane Database Syst Rev* 2005; (3): CD004954
70 Hypothermia after Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *N Engl J Med* 2002; **346**: 549–556
71 Bernard SA, Gray TW, Buist MD, *et al.* Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002; **346**: 557–563

72 Rosenthal RE, Silbergleit R, Hof PR, *et al.* Hyperbaric oxygen reduces neuronal death and improves neurological outcome after canine cardiac arrest. *Stroke* 2003; **34**: 1311–1316
73 Takahashi M, Iwatsuki N, Ono K, *et al.* Hyperbaric oxygen therapy accelerates neurologic recovery after 15-minute complete global cerebral ischemia in dogs. *Crit Care Med* 1992; **20**: 1588–1594
74 Krakovsky M, Rogatsky G, Zarchin N, *et al.* Effect of hyperbaric oxygen therapy on survival after global cerebral ischemia in rats. *Surg Neurol* 1998; **49**: 412–416
75 Mickel HS, Kempski O, Feuerstein G, *et al.* Prominent white matter lesions develop in mongolian gerbils treated with 100% normobaric oxygen after global brain ischemia. *Acta Neuropathol (Berl)* 1990; **79**: 465–472
76 Mickel HS, Vaishnav YN, Kempski O, *et al.* Breathing 100% oxygen after global brain ischemia in mongolian gerbils results in increased lipid peroxidation and increased mortality. *Stroke* 1987; **18**: 426–430
77 Vereczki V, Martin E, Rosenthal RE, *et al.* Normoxic resuscitation after cardiac arrest protects against hippocampal oxidative stress, metabolic dysfunction, and neuronal death. *J Cereb Blood Flow Metab* 2005; **26**: 821–835
78 Liu Y, Rosenthal RE, Haywood Y, *et al.* Normoxic ventilation after cardiac arrest reduces oxidation of brain lipids and improves neurological outcome. *Stroke* 1998; **29**: 1679–1686
79 Zwemer CF, Whitesall SE, D'Alecy LG. Cardiopulmonary-cerebral resuscitation with 100% oxygen exacerbates neurological dysfunction following nine minutes of normothermic cardiac arrest in dogs. *Resuscitation* 1994; **27**: 159–170
80 Zwemer CF, Whitesall SE, D'Alecy LG. Hypoxic cardiopulmonary-cerebral resuscitation fails to improve neurological outcome following cardiac arrest in dogs. *Resuscitation* 1995; **29**: 225–236
81 Agardh CD, Zhang H, Smith ML, *et al.* Free radical production and ischemic brain damage: Influence of postischemic oxygen tension. *Int J Dev Neurosci* 1991; **9**: 127–138
82 Mink RB, Dutka AJ. Hyperbaric oxygen after global cerebral ischemia in rabbits does not promote brain lipid peroxidation. *Crit Care Med* 1995; **23**: 1398–1404
83 Lipinski CA, Hicks SD, Callaway CW. Normoxic ventilation during resuscitation and outcome from asphyxial cardiac arrest in rats. *Resuscitation* 1999; **42**: 221–229
84 Shiokawa O, Fujishima M, Yanai T, *et al.* Hyperbaric oxygen therapy in experimentally induced acute cerebral ischemia. *Undersea Biomed Res* 1986; **13**: 337–344
85 Piantadosi CA, Zhang J. Mitochondrial generation of reactive oxygen species after brain ischemia in the rat. *Stroke* 1996; **27**: 327–331
86 Elayan IM, Axley MJ, Prasad PV, *et al.* Effect of hyperbaric oxygen treatment on nitric oxide and oxygen free radicals in rat brain. *J Neurophysiol* 2000; **83**: 2022–2029
87 Oter S, Korkmaz A, Topal T, *et al.* Correlation between hyperbaric oxygen exposure pressures and oxidative parameters in rat lung, brain, and erythrocytes. *Clin Biochem* 2005; **38**: 706–711
88 Jenkinson SG. Oxygen toxicity. *New Horiz* 1993; **1**: 504–511
89 Lodato RF, Jubran A. Response time, autonomic mediation, and reversibility of hyperoxic bradycardia in conscious dogs. *J Appl Physiol* 1993; **74**: 634–642
90 Lodato RF. Decreased O_2 consumption and cardiac output during normobaric hyperoxia in conscious dogs. *J Appl Physiol* 1989; **67**: 1551–1559
91 Watson NA, Beards SC, Altaf N, *et al.* The effect of hyperoxia on cerebral blood flow: A study in healthy volunteers using magnetic resonance phase-contrast angiography. *Eur J Anaesthesiol* 2000; **17**: 152–159
92 Nakajima S, Meyer JS, Amano T, *et al.* Cerebral vasomotor responsiveness during 100% oxygen inhalation in cerebral ischemia. *Arch Neurol* 1983; **40**: 271–276
93 Dani C, Cecchi A, Bertini G. Role of oxidative stress as physiopathologic factor in the preterm infant. *Minerva Pediatr* 2004; **56**: 381–394
94 Tan A, Schulze A, O'Donnell CP, *et al.* Air versus oxygen for resuscitation of infants at birth. *Cochrane Database Syst Rev* 2005; (2): CD002273
95 Dembinska O, Rojas LM, Varma DR, *et al.* Graded contribution of retinal maturation to the development of oxygen-induced retinopathy in rats. *Invest Ophthalmol Vis Sci* 2001; **42**: 1111–1118
96 Calvert JW, Zhou C, Zhang JH. Transient exposure of rat pups to hyperoxia at normobaric and hyperbaric pressures does not cause retinopathy of prematurity. *Exp Neurol* 2004; **189**: 150–161
97 Forman HJ, York JL, Fisher AB. Mechanism for the potentiation of oxygen toxicity by disulfiram. *J Pharmacol Exp Ther* 1980; **212**: 452–455
98 Monstrey SJ, Mullick P, Narayanan K, *et al.* Hyperbaric oxygen therapy and free radical production: An experimental study in doxorubicin (adriamycin) extravasation injuries. *Ann Plast Surg* 1997; **38**: 163–168
99 Jamieson DD, Kerr DR, Unsworth I. Interaction of n-acetylcysteine and bleomycin on hyperbaric oxygen-induced lung damage in mice. *Lung* 1987; **165**: 239–247
100 Mink RB, Dutka AJ. Hyperbaric oxygen after global cerebral ischemia in rabbits reduces brain vascular permeability and blood flow. *Stroke* 1995; **26**: 2307–2312
101 Sukoff MH, Hollin SA, Espinosa OE, *et al.* The protective effect of hyperbaric oxygenation in experimental cerebral edema. *J Neurosurg* 1968; **29**: 236–241
102 Sukoff MH. Effects of hyperbaric oxygenation. *J Neurosurg* 2001; **95**: 544–546
103 Murakami N, Horinouchi T, Sakurai M, *et al.* Hyperbaric oxygen therapy given 30 minutes after spinal cord ischemia attenuates selective motor neuron death in rabbits. *Crit Care Med* 2001; **29**: 814–818
104 Lim J, Lim WK, Yeo TT, *et al.* Management of haemorrhagic stroke with hyperbaric oxygen therapy – a case report. *Singapore Med J* 2001; **42**: 220–223
105 Kohshi K, Yokota A, Konda N, *et al.* Hyperbaric oxygen therapy adjunctive to mild hypertensive hypervolemia for symptomatic vasospasm. *Neurol Med Chir (Tokyo)* 1993; **33**: 92–99
106 Ostrowski RP, Colohan AR, Zhang JH. Mechanisms of hyperbaric oxygen-induced neuroprotection in a rat model of subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2005; **25**: 554–571
107 Singhal AB, Wang X, Sumii T, *et al.* Effects of normobaric hyperoxia in a rat model of focal cerebral ischemia-reperfusion. *J Cereb Blood Flow Metab* 2002; **22**: 861–868
108 Kim HY, Singhal AB, Lo EH. Normobaric hyperoxia extends the reperfusion window in focal cerebral ischemia. *Ann Neurol* 2005; **57**: 571–575
109 Flynn EP, Auer RN. Eubaric hyperoxemia and experimental cerebral infarction. *Ann Neurol* 2002; **52**: 566–572
110 Hempel FG, Jobsis FF, LaManna JL, *et al.* Oxidation of cerebral cytochrome aa3 by oxygen plus carbon dioxide at hyperbaric pressures. *J Appl Physiol* 1977; **43**: 873–879
111 Ronning OM, Guldvog B. Should stroke victims routinely receive supplemental oxygen? A quasi-randomized controlled trial. *Stroke* 1999; **30**: 2033–2037
112 Kidwell CS, Saver JL, Mattiello J, *et al.* Thrombolytic reversal of acute human cerebral ischemic injury shown by diffusion/perfusion magnetic resonance imaging. *Ann Neurol* 2000; **47**: 462–469
113 Fisher M. Recommendations for advancing development of acute stroke therapies: Stroke Therapy Academic Industry Roundtable 3. *Stroke* 2003; **34**: 1539–1546
114 Kidwell CS, Liebeskind DS, Starkman S, *et al.* Trends in acute ischemic stroke trials through the 20th century. *Stroke* 2001; **32**: 1349–1359