

# Hyperbaric oxygen modalities are differentially effective in distinct brain ischemia models

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## Abstract

The effectiveness and efficacy of hyperbaric oxygen (HBO) preconditioning and post-treatment modalities have been demonstrated in experimental models of ischemic cerebrovascular diseases, including global brain ischemia, transient focal and permanent focal cerebral ischemia, and experimental neonatal hypoxia-ischemia encephalopathy. In general, early and repetitive post-treatment of HBO appears to create enhanced protection against brain ischemia whereas delayed HBO treatment after transient focal ischemia may even aggravate brain injury. This review advocates the level of injury reduction upon HBO as an important component for translational evaluation of HBO based treatment modalities. The combined preconditioning and HBO post-treatment that would provide synergistic effects is also worth considering.

**Key words:** hyperbaric oxygen; cerebral ischemia; brain injury; infarction volume; cell death; hippocampus; therapeutic window; preconditioning; animal models

**doi:** 10.4103/2045-9912.179344

**How to cite this article:** Ostrowski RP, Stępień K, Pucko E, Matyja E (2016) Hyperbaric oxygen modalities are differentially effective in distinct brain ischemia models. *Med Gas Res* 6(1):39-47.

## INTRODUCTION

Hyperbaric oxygenation is the method to assist in improving outcomes of the treatment for stroke and global brain ischemia. When patients undergo hyperbaric oxygen therapy, they breathe 100% oxygen under greater than sea level pressure. This modality is capable of providing maximized oxygen supply due to hyperbaric conditions while oxygen deficiency underpins the development of brain damage after stroke.

The ischemic stroke accounts for approximately 80% of strokes (Moretti et al., 2015). It is most often caused by the occlusion of blood vessels, that provide blood to the brain, due to atherosclerotic plaque enlargement or through the formation of a clot on atherosclerotic alterations possibly with a subsequent distal dislodgement (15–30% of ischemic strokes) or caused by emboli from distinct parts of circulation, mainly from the heart valves and chambers (20–25% of ischemic strokes) (Wiebers et al., 2006). While, global brain ischemia, differentiated from focal ischemic stroke,

predominately affects selectively vulnerable anatomical regions within the brain and can be caused by cardiac arrest or near-drowning incidents. Cerebral ischemic injury may be also formed during hypoxic-ischemic events in the newborns (Wang et al., 2014).

Apart from unclear treatment mechanisms, a limited effectiveness of preclinical therapies is one of the major factors precluding progress towards their clinical implementation against brain injuries. The ultimate hyperbaric oxygen (HBO) effectiveness determination at the preclinical stages might offer a prediction which groups of patients might benefit most from HBO therapy, thereby increasing the therapeutic success in clinical trials. In this context, the neuroprotective effects of HBO against brain ischemia at the histological level was detected and was adopted as a leading subject of this review.

The question arises as to why HBO is being considered as optimal therapeutic option to treat cerebral ischemic injury rather than normobaric oxygen (NBO) (Weaver and Liu, 2015). Under ischemic conditions, NBO may



not be able to provide sufficient oxygen to the nervous tissue while HBO can (Sun et al., 2008). In general, HBO treatment can produce better outcomes than NBO in many studies of experimental brain injury (Beynon et al., 2007; Eschenfelder et al., 2008; Huang and Obenaus, 2011). In addition, one study has shown that hyperbaric oxygen may produce more consistent protection than hyperbaric air pressure after 1-hour focal ischemia, since HBO improved all indices of brain injury, while treatment with hyperbaric air ameliorated only selected parameters of brain damage (Chang et al., 2000).

To determine effectiveness and efficacy of HBO, animal models of CNS disease are required. Animal models and not cell cultures can replicate pathophysiological phenomena of brain injury. The alleviation of cerebral pathophysiological changes by HBO is pivotal for producing brain protective effect. Rodent focal and global cerebral ischemia belongs to most common models of brain injuries used to study therapeutic effects of HBO. However direct comparisons of HBO effects across different models of ischemia are nearly impossible. Quantitative indicators of brain injury are different between focal and global cerebral ischemia (e.g., infarct volume for focal ischemia *versus* damaged hippocampal cell count for global cerebral ischemia). These parameters cannot be compared, however, percent reductions of injuries produced by the HBO treatment may provide a broad assessment of its effectiveness. In addition, the effectiveness of HBO preconditioning and HBO post-treatment can be compared in terms of their effects on the same characteristics of brain injury, e.g., infarct volume. However, differences in experimental settings render such comparisons approximative most of the time.

### **HBO FOR GLOBAL CEREBRAL ISCHEMIA**

In the global brain ischemia, both post-treatment and preconditioning with HBO as a procedure increasing the resistance of cells against ischemic injury was examined. Patients that may experience generalized ischemia can be selected beforehand: those are endangered with circulatory arrest or subjected to long lasting complex surgeries e.g., with cardiopulmonary bypass when there is a risk of hypoperfusion in the cerebral circulation. Effectiveness of HBO treatment was verified in a rat model of 10 minutes of global cerebral ischemia *via* blood pressure (BP) reduction to 30–35 mmHg and bilateral common carotid artery occlusion (Li et al., 2005). HBO at 3 atmospheres absolute (ATA) for 2 hours at 1 hour after ischemia can result in 50.5% reduction of cell loss in hippocampal CA1 at 7 days after ischemia. Earlier, at 96 hours the 57.1% reduction of cell loss occurred while at 48 hours 59.6% reduction of cell loss was found following the hyperbaric oxygen treatment.

At 96 hours and 7 days, HBO reduced cell loss by 34.2% and 33% in the cerebral cortex in rats with global ischemia, respectively (Zhou et al., 2003).

A short time to treatment is important to the effectiveness of HBO. HBO was shown to increase neuronal survival rate in the hippocampal area CA1 and the amelioration of neurologic deficits after 5 minutes of global brain ischemia in gerbils (Malek et al., 2013). 60 minutes of 2.5 ATA HBO treatment applied at 1 hour after ischemia repeated for 3 days subsequently reduced global cerebral ischemia-induced neuronal damage in the hippocampal area CA1 by 43.9%. However, HBO initiated 3–6 hours after ischemia reduced cell loss in the hippocampus by 28.0% after ischemia. Both repetitive HBO treatment and a short delay to HBO treatment appear to increase HBO effectiveness against global brain ischemia.

However, despite obvious indispensability of treatment post ischemia, there may be a clinical justification of HBO preconditioning (HBO-PC) approach. Increasing the resilience of ischemically challenged cells may turn out to be a more effective strategy than to cure patients with completed brain damage. Besides, the effectiveness and underlying mechanisms of HBO preconditioning need to be examined to avoid unwanted interactions of HBO with administered medications. In order to provide a rationale for a preconditioning approach one can demonstrate the appearance of brain injury markers very early after ischemia and argue that post-treatment approach can usually be started only after this initial injury occurs. In particular, early apoptosis is observable already after 3 hours from the ischemic event and is marked with appearance of phosphatidylserine residues on the outer leaflet of the cell membrane. In one study, the preconditioning with HBO was conducted with two distinct regimes of oxygenation (both at 2.5 ATA): 5 treatments with one treatment per day; the last HBO session at 24 hours before ischemia (5HBO); or 3 treatments within 24 hours (3HBO): at 24, 12 and 4 hours prior to 10 minute global ischemic insult induced by four-vessel occlusion. After this ischemia, 32.1% of all cells in the cerebral cortex became apoptotic prior to 3 hours post-ischemia time point. When comparing 3HBO protocol to 5HBO, a higher efficacy of 5HBO was found, based on 81.5% reduction in early apoptotic cell count compared to 69.1% reduction with 3HBO regimen. This substantial reduction of early brain injury appears to favor preconditioning paradigm with HBO for a global brain ischemia. However, the reduction of delayed brain injury with HBO-PC appears more modest. It is nevertheless impressive that HBO (5 days of 1 hour-long sessions at 2.5 ATA daily) can reduce cell death within CA1 hippocampal region after a relatively long, 15 minute global brain ischemia (Cheng et al., 2011). Seven days after ischemia, 85.1% damage of CA1 neurons was noted in unpreconditioned group versus 58.8% in hy-



perbaric oxygen preconditioned group, what denotes 30.9% damage reduction rate.

Then the question arises whether increasing hyperbaria upon preconditioning procedure for a global brain ischemia will translate into an improved neuroprotective effect. In the study of Hirata et al. (2007), HBO-PC at 3.5ATA (1 hour daily for 5 days) reduced CA1 cell loss after 8 min of forebrain ischemia (Hirata et al., 2007). Depending on whether the last HBO session was given at 6, 12 or 24 hours before ischemia, cell loss reduction was by 54.5%, 74.7% or 52.5%, respectively, compared to 99% loss in unpreconditioned group at 7 days after ischemia. Although these results appear quite impressive, 3.5 ATA may not be well tolerated by all patients in the clinic. In addition, even when hyperbaria is moderate, neuroprotection against global brain ischemia may be obtained with sophisticated preconditioning regimen such as in the study of Wada et al. Preconditioning with HBO at 2 ATA once every other day for three or five sessions (but not one session), resulted in 20.3% and 49.6 % reductions of neuronal loss at CA1, respectively, as determined 7 days after 5-minute global ischemia in gerbils (50.9% reduction in another study from the same research group). Such efficacy is comparable with the one obtained in the most efficient repetitive HBO post treatment regimens. One hour HBO at 3 ATA, once daily for 10 sessions prior to ischemia did not induce ischemic tolerance (Wada et al., 2001). Thus, beside hyperbaria parameters, elaborate preconditioning regimen with optimal anticipation interval to a global brain ischemia may contribute to neuroprotection.

In conclusion, the enhanced hyperbaria for HBO preconditioning and rather moderate pressures for post-treatments, so as to not aggravate brain injury-induced oxidative stress, both are the factors that may contribute to the therapeutic efficacy of HBO for global ischemia. Another question then arises if pretreatment with HBO is possible with sophisticated regimen or combined modalities, which all require further study.

## **HBO FOR TRANSIENT FOCAL CEREBRAL ISCHEMIA**

Partial histological neuroprotection with HBO after global ischemia is somewhat intriguing given that HBO applied after transient focal ischemia may result in substantial reduction of cerebral infarcts formed after relatively long periods of vessel occlusion. Several studies used HBO with different delays after transient middle cerebral artery occlusion (MCAO). Three atm HBO, administered twice for 90 minutes with 24-hour interval, and started immediately or 1 hour after 60 min brain ischemia in Sprague Dawley (SD) rats brought a reduction in cerebral infarction volume by 24 hours after ischemia. While hyperbaric air (HBA) started im-

mediately after ischemia reduced infarction as well, 1 hour delay to HBA treatment was not associated with such effect (Chang et al., 2000). In another study, 1 hour HBO at 3 ATA, initiated at 3 or 6 hours after the onset of ischemia, but not 12 hours, was neuroprotective against 90-minute MCAO in SD rats (Lou et al., 2004). Total infarct volumes were reduced by 70.2% with HBO applied at 3 hours post stroke, and 45.1% when HBO was applied at 6 hours. And although cortical infarct volumes were reduced by 91% and 50.8%, however, in striatal region only 33.3% reduction at 3 hours and 30.4% reduction at 6 hours were noted. On day 7, histological analysis revealed 54% and 52% total infarct volume reductions following 3 and 6 hour delays to HBO, respectively. In addition, when 120 minutes MCAO in SD rats was treated with 3 ATA HBO for 1 hour, applied at 3 or 6 hours but not at 12 hours post ischemia, it reduced the infarction area by 33.1% and 52.8% respectively, 24 h after reperfusion. When the treatment started at 12 hours, it even increased infarction by 102.8% (Badr et al., 2001). Consistent with the above report, hyperbaric oxygen therapy (2.5 ATA for 2 hours) at 6 hours after reperfusion in SD rats with 120-minute MCAO, reduced the infarct area by 37.3% and improved neurologic scores at 7 days after reperfusion (Yin et al., 2003).

At the same time multiple treatments appear of greater benefit than 1–2 treatments for transient MCAO. In one study of 2 HBO treatments daily, with 1 hour of HBO at 2ATA each, the first treatment started 3 hours after 90-minute MCAO. This regimen was used in rats for 2–4 days. Three or four day course treatment of HBO caused a significantly reduced infarction volume on day 7 as compared to 2-day length of HBO (Chen et al., 2014). However for permanent MCAO, a single treatment may provide advantage over repeated ones. A single 90-minute HBO at 2.5ATA when used since 15, 90 or 180 minutes after permanent MCAO in rats, significantly decreased infarct volume, by 24%, 16% and 13%, respectively, 7 days after ischemia. In contrast, repetitive HBO treatment started 90 minutes after permanent MCAO was ineffective, as was a single treatment delayed till 360 minutes post MCAO (Gunther et al., 2005).

Interesting data were obtained when HBO was applied during the occlusion of cerebral arteries. In mice with 90-minute MCAO, 3 ATA HBO for 1 hour intraschemically, reduced infarct volume by 49.1%, as determined at 24 hours after MCAO (Sun et al., 2014). Likewise, HBO at 2.8 ATA for 1 hour during 60-minute MCAO reduced the lesion volume by 38.9%, as found 2 hours after reperfusion in SD rats (Yang et al., 2010). Also, HBO at 3 ATA of 100 minutes duration, was started at 40 minutes after the onset of 120-minute MCAO in Wistar rats, which brought 41.5% histological infarct reduction, as calculated after edema correction 24 hours post ischemia. On MRI scan in the treatment group, the area of



T2 hyper intensity was reduced to a similar degree at this time point (40%) (Veltkamp et al., 2005a).

Even in the study of 150 minute-long MCAO in Wistar rats, 3 ATA HBO of 60 minutes duration, started 90 minutes after the beginning of MCAO, reduced the infarct volume by 30.7% after 7 days. Clearly HBO applied prior to reperfusion can be effective with such substantial MCAO duration. On the other hand NBO of 300-minute duration, even initiated 30 minutes after start of 150-minute MCAO failed to decrease infarct volume significantly (Beynon et al., 2007). When HBO treatment is carried out during ischemia, it also reduces blood-brain barrier (BBB) disruption. Mean abnormal enhancing volume on T1, the postcontrast surrogate of BBB damage, showed a maximal, 43.5% reduction at 6 hours after 120-minute MCAO, when 3ATA HBO was conducted for 1 hour during the occlusion in Wistar rats (Veltkamp et al., 2005b). This is an important finding, since early reports claimed that despite the capability to reduce brain edema, HBO may increase BBB permeability in normal conditions (Lanse et al., 1978).

In addition to the therapeutic time window for HBO, various hyperbaric pressures were also investigated. In the study by Veltkamp et al., cerebral ischemia due to 75 minute-MCAO was treated during occlusion with 1-hour HBO at 1.5 or 2.5 ATA, which reduced infarction volumes by 7.8% and 41.1%, respectively, 7 days after ischemia comparing to the control with NBO (100% O<sub>2</sub>) (Veltkamp et al., 2000). When 1-hour HBO was applied 90 minutes after the end of 90-minute MCAO, out of 1.5, 2.0, 2.5 and 3.0 ATA hyperbaric pressures, the last two proved to provide lasting protection, reflected by 58.7% and 63.3 % reductions of total infarct volumes on day 7, respectively. At that time point, even reductions of striatal injuries were observed with 2.5 and 3 ATA HBO treatments, around 58% at both levels of hyperbaria. Thus not only an early initiation but also an application at high doses may render O<sub>2</sub> a neuroprotective molecule (Eschenfelder et al., 2008).

More recently it was revealed that the combination of HBO and pharmacological agents may further increase the infarct reduction rates. One hour of HBO at 3 ATA or c-Jun N-terminal kinase (JNK) inhibitor XG-102 were applied 3 hours after the onset of 90-minute MCAO. At 24 hours, total cerebral infarct area was reduced with JNK inhibitor or HBO alone by 43% and 63%, respectively. Of note, when HBO was combined with JNK inhibitor XG-102, the reduction increased to 78%, significantly over the effect of XG-102 treatment alone (Liu et al., 2010). While, 90 minutes of MCAO in Wistar rats treated with HBO at 3 ATA for 1 hour at 3 hours after reperfusion even increased the infarct size as determined with TTC 24 hours after reperfusion. In addition, a disrupted autophagy flux was noted (Lu et al., 2014). Although the start of HBO

therapy at 3 hours after reperfusion might appear inside the therapeutic window, it may only be true for studies with SD rats while differential effects of HBO can be strain specific. Far from being a cautionary tale, these reports may indicate a need for HBO studies to enter the personalized medicine arena.

## HBO FOR PERMANENT FOCAL CEREBRAL ISCHEMIA

As already mentioned above, permanent focal ischemia was also investigated with regard to HBO treatment efficacy. Administering HBO (2.5 ATA for 1 hour) at 3 hours after permanent focal brain ischemia reduced the extent of infarct size by 52.3%, 24 hours post occlusion, and ameliorated neurobehavior (Mu et al., 2013). In the study of permanent MCAO combined with 60 minutes of bilateral common carotid artery occlusion in SD rats, 1 hour of HBO at 3 ATA was administered just before surgery or right after. Both pretreatment and post-treatment reduced infarction volumes, by 30.8 and 28.2%, respectively, at 24 hours after MCAO (Miljkovic-Lolic et al., 2003). Permanent middle cerebral artery occlusion in Wistar rats treated with 2 atm HBO 2 hours after ischemia yielded a reduced infarct size by 16.7% as measured 24 hours post ischemia, and by 23.9% on the fifth postischemic day. Additionally, after 5 days TTC showed the reduction in brain infarct by 32.8% with HBO (Schabitz et al., 2004). Here also belongs the study of Sunami et al. (2000), where 2 hours of HBO at 3 ATA was initiated 10 minutes after the beginning of permanent MCAO in rats, what brought 18% infarct reduction rate 24 hours after the onset of ischemia. In view of these data it seems that early HBO treatment for permanent ischemia allows more modest infarct reduction rates as compared to those obtained for transient ischemia.

In addition, considering difficulties with administering HBO in the early time period post stroke, a delayed treatment should be tested. However, several authors assumed that HBO administered in permanent MCAO beyond 6h therapeutic window will fail to reduce the infarction volume. In their experiments, total brain infarction volumes after permanent focal ischemia in rats were not reduced significantly with 1 hour of HBO at 3 ATA, neither with 3 hours nor 6 hours delays to treatment (8% and 22% insignificant reductions, respectively) (Lou et al., 2004). However, quite different outcomes emerge from the study by Mu and coworkers (2013) who used the permanent focal ischemia model. HBO was administered at 48 hours after permanent ischemia for 1 hour daily at 2.5 ATA for a total of 10 days. As determined on days 14 and 28, HBO reduced the extent of infarction by 57.7% and 60.7%, respectively, and ameliorated neurological and cognitive deficits. In addition, HBO increased cell proliferation in the regions of ischemic



brain injury. The results of these experiments may indicate a validity of delayed administration of hyperbaric oxygen in order to activate regenerative mechanisms of the brain and to improve neurological function after stroke (Mu et al., 2013).

### **HBO PRECONDITIONING FOR FOCAL BRAIN ISCHEMIA**

HBO was also applied prior to focal cerebral ischemia as a preconditioning modality. It needs to be mentioned though, that with respect to preconditioning for focal ischemia literature data are somewhat conflicting. Some studies claim that HBO preconditioning is protective only towards transient brain ischemia while others show its effectiveness only in permanent but not transient ischemia (Prass et al., 2000; Xiong et al., 2000). One hour of daily HBO treatment at 2.5 ATA, for 3 or 5 days, was used in a rat prior to 120-minute or permanent MCAO followed by examinations 24 hours after ischemia. HBO preconditioning for 3 and 5 days reduced cerebral infarctions, by 76.3% and 90.6%, respectively, in transient MCAO group but not in permanent MCAO group (Xiong et al., 2000). Although no one else reported 90% infarct reduction rates with the same regimen, this result might have been indicative of clinical relevance. Li et al. (2011) used 5 × HBO at 2 ATA preconditioning protocol and found the amelioration of biochemical markers of neuronal and myocardial injury as well as improved clinical outcomes in on-pump coronary artery bypass graft (CABG) patients postsurgically. Other researchers successfully used HBO at 2.4 ATA thrice within 24 hours as a preconditioning protocol in patients with on-pump CABG, what led to a reduced neuropsychometric dysfunction and inflammatory response after cardiopulmonary bypass (CPB) (Alex et al., 2005). Other HBO protocols and treatment modifications that may appear promising were tested for effectiveness upon laboratory investigations. One study used HBO at 2.5 ATA every 12 hours for 2 days (4 treatments) prior to 90-minute MCAO. At 24 hours, cell counts of injured neurons in the penumbra and in the hippocampus were significantly reduced (Li et al., 2008a). Interestingly, the effectiveness of HBO-PC in transient ischemia may depend upon autophagy activation, as determined in SD rats with 120 minutes of MCAO, preconditioned with HBO 2.5 ATA 1 hour daily for 5 days (Yan et al., 2011).

In contrast, 1 hour of HBO-PC at 3 ATA for 5 days in SV129 mice caused the infarct size reduction by 27.15% after permanent ischemia, but not after transient ischemia as measured after 24 and 72 hours, respectively. It was suggested that some factors released during reperfusion may overwhelm or abolish preconditioning effect (Prass et al., 2000). Experimental studies of preconditioning, however, clearly show the advantage of hyperbaric oxygen

over oxygen in normobaric conditions. Zhang et al. (2004) showed that it took 24-hour breathing normobaric 100% oxygen by SD rats for 47% reduction of infarct volume 24 hours after 120-minute MCAO.

Several studies of HBO investigated focal cerebral ischemia induced by perivascular administration of ET-1, adjacent to MCA. In one study, the activation of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) was demonstrated with HBO-PC alone (the increase of protein level and transcriptional activity) combined with the increase of HIF-1 dependent gene, erythropoietin (EPO), in the cerebral cortex and hippocampus. While, after ET-1-induced focal brain ischemia in rats preconditioned with HBO, there was reduced brain tissue damage by 39.6% and a decrease of neurological deficit 24 hours after ET-1 injection (Gu et al., 2008). ET-1-induced ischemia was subject to HBO post treatment as well. HBO at 2.5 ATA for 2 hours was initiated at 1 hour after ET-1-induced cerebral ischemia in rats. At 24 hours later, a remarkable 59.6% reduction of infarct size was noted with HBO treatment (Huang et al., 2007).

### **HBO FOR HEMORRHAGIC TRANSFORMATION**

The effect of HBO on hemorrhagic transformation in focal cerebral ischemia was studied for both treatment with HBO and HBO preconditioning approach. SD rats received HBO at 3 ATA for 1 hour, 30 minutes after start of occlusion (120 minutes of MCAO), which reduced mean infarct volume in the basal ganglia by 37.2%, at 24 hours after MCAO. Hemorrhagic transformation, as determined by brain Hb content, was reduced by 58.1% (Qin et al., 2007). Another study used HBO preconditioning (2.5 ATA, 1 hour/day for 5 days) for a focal cerebral ischemia complicated by hemorrhagic transformation (Soejima et al., 2013). This experimental model is based on 2-hour MCAO, combined with hyperglycemia induced by the administration of 50% dextrose (Soejima et al., 2013). At 24 hours after ischemia induction HBO-PC only tended to reduce the extent of brain infarction, while it reduced hemorrhagic transformation more than half. Hyperbaric oxygen preconditioning did not have any effect on the level of glucose, measured prior to and after dextrose application. Thus, it seems that HBO-PC acts specifically towards amelioration of BBB disruption after MCAO, possibly *via* inhibiting matrix metalloproteinases (Soejima et al., 2013). However, since the predictability of stroke occurrence is limited, HBO-PC procedure may need to be redesigned to a chronic conditioning in order to induce a long lasting resilience of brain to injury. Further studies of distinct mechanisms of chronic conditioning are also warranted (Zhu et al., 2015).

### **NEONATAL HYPOXIA-ISCHEMIA (HI) AND USE OF HBO**

HBO treatment was applied in the studies of experimental



neonatal hypoxia-ischemia encephalopathy (HIE). Calvert et al. (2002) found, that in SD P7 rats subjected to 2.5 hours of hypoxia treated with HBO at 3 ATA 1h after hypoxia exposure, the attenuations of hemispheric weigh reductions were 53.1% and 62.7% at 2 weeks and 6 weeks after hypoxia, respectively (Calvert et al., 2002). Studies have also demonstrated an optimized benefit from the early intervention. P7 SD rats with 2-hour-long hypoxia-ischemia were subjected to 60 minutes of HBO under 2.0 ATA at 3, 6, 12, 24, and 72 hours after hypoxia. Then Nissl stain was conducted 28 days after HI showing that HBO applied at 3, 6 and 12 hours lessened the neuronal loss in the hippocampal CA1 by 89.9%, 83.7%, and 69.2%, respectively. While HBO, started at 24 or 72 hours after HI, produced insignificant effects with this regard (Wang et al., 2008). Gamdzyk et al. (2013) confirmed that early treatment gives better results for brain protection and they also proved a superiority of HBO over other pressure modulation-based preconditioning modalities. They observed that HBO at 2.5 ATA repeated for three days reduced brain damage by 58.1%, 57.6% and 54.9%, with 1, 3, 6 hours of delay to treatment, respectively, after 75-minute HI. While, hyperbaric air decreased the damage by 29.9%, 38.1% and 22.0%, respectively whereas hypobaric hypoxia by 66.1%, 39.2% and 37.4%, with respect to the 1, 3, and 6 hours delay of treatment initiation. Based on their study, there is also an indication that a repetitive treatment is worth considering for optimal outcomes. Other studies indicate that the protective effect of HBO in the newborn is long lasting. P7 rat pups after 120-minute HI were subjected to HBO at 2.5 ATA for 90 minutes, starting 1 hour after HI. In P60 rats from the treatment group, mean neuropathological lesion reductions were 24.5% and 29.8% in the cerebral cortex and in the hippocampus, respectively, when compared with untreated HI group (Liu et al., 2013). The extent of injury reduction with HBO appears consistent across neonatal studies. The common use of quite well standardized Rice-Vanucci model of HI may contribute to this consistency (Rice et al., 1981).

Interesting data originate from HBO preconditioning studies for the newborn, showing a high effectiveness of this modality. In P7 rat pups 8% O<sub>2</sub> administered for 90 minutes, was preceded by a single 150 minutes of HBO treatment at 2.5 ATA, 24 hours prior to HI. While 7 days after untreated HI the infarct ratio was 10.5%, HBO-PC reduced the injury by 91.7% to 0.9% (Li et al., 2008b).

Interestingly, both HBO preconditioning and hypoxic preconditioning elicited similar neuroprotective efficacy in the neonatal brain (Freiberger et al., 2006). However, as neonatal brain is more resistant to injury, a potent preconditioning inducer can be required, which may favor HBO-PC, that appears clinically safer. Although the results are promising, oxygen-induced retinopathy remains the major

limitation and concern. However, a transient single exposure to hyperoxia does not appear to disturb retinal vascularization. A sudden withdrawal from the high level of oxygen may be a real factor behind vision disturbances and there is no rationale for completely withholding oxygen therapy in the neonatal period (Calvert et al., 2004).

This therapeutic success of HBO in preclinical studies of neonatal brain injury may relate to its specific interference with HIF-1 pathway. HIF-1 $\alpha$  can be involved both in the mechanisms of hypoxic brain injury and the adaptation of nervous system to perinatal hypoxia. Comparing to the mature brain, carrying out adaptive hypoxic responses in the newborn can be facilitated by a higher basal HIF-1 activation. This may also translate to a known higher resistance of neonatal brains to hypoxia (Chen et al., 2009). Although it requires further study, the potent HIF-1 activation in the neonatal brain upon hypoxia, may allow HBO to reduce only HIF-1 excess and thus not to completely abolish adaptive functions of HIF-1 pathway.

## CONCLUSIONS, LIMITATIONS AND FINAL REMARKS

Based on the present review we conclude that the effectiveness and efficacy of HBO modalities may vary upon type of ischemia, and treatment regimen characteristics. This present analysis has found one of the highest percent injury reductions with the preconditioning prior to a transient focal cerebral ischemia and neonatal HIE. However, it also appears that highly remarkable brain injury reductions with HBO might be obtained in nearly all studied models of injury under certain conditions. In general, early and repetitive post-treatment may enhance protection against brain ischemia. However, a delayed treatment after transient focal cerebral ischemia may aggravate brain injury. There seems to be an exception for permanent brain ischemia, where delayed treatment may even surpass early intervention in terms of efficacy. Increasing hyperbaric pressures for preconditioning may provide for another direction to be considered, especially for a global ischemia.

However, the present review also carries certain limitations. As a regular literature review and not a systematic review it may miss portions of data regarding the subject, despite being based on authors' knowledge and research done on hyperbaric oxygen during over the 10-year span, and widespread database searches including relevant key words. Also, even though this review takes into account only peer-reviewed research, studies of different qualities have been included in order to arrive at conclusions. Therefore, those have been cautiously stated.

The results of several studies have shown that investigating novel treatment regimens and combined therapies can form promising research directions. The interesting issue is that combined HBO preconditioning and HBO



post-treatment might provide synergistic neuroprotective effects. Even if both modalities share some signal transduction pathways intracellularly, their timely separation might still enable synergistic protection (Jiang et al., 2014). Another question remains as to whether these studies successfully observe postulates from prominent scientists who advocate more rigorous research. Although studies can be classed and analyzed according to their level of confidence, it seems that definite judgment should be withdrawn at this point and promising experimental results in terms of high efficacy, although with some methodological insufficiencies, should be additionally verified in order not to overlook any prospective treatment options.

In the recent years the knowledge about mechanisms of HBO for stroke has increased, which enabled (together with a better availability of the sophisticated neurointensive care equipment) an improved management of patients in stroke care units. However, there are few therapeutic agents combating stroke with the effectiveness proven in multicenter clinical trials (Lapchak, 2015). Such study for HBO is warranted. Preclinical translational research needs to further identify conditions in which HBO may exert robust effects of neuroprotection and neurorepair. In order to do this, percent reduction of brain injury should be calculated with scrutiny at distinct time points after ischemia. Investigating efficacy of HBO-based therapies in diverse models of ischemia may provide an insight as to which ischemic conditions might benefit most from HBO therapy. In addition, since the salvaged tissue may include neural and angiogenic endogenous progenitors, a great efficacy of a given treatment may help predict the involvement of neuro- and angiogenesis in the brain repair process.

Following escalation of STAIR criteria, investigational treatments are expected to fulfill RIGOR guidelines in order to advance into clinical trials (Lapchak et al., 2013). The extent of injury reduction in cerebrovascular disease models should be also taken into account before experimental therapies are considered clinically.

#### Author contributions

RPO initialed the topic and wrote the first draft of the manuscript, KS helped to draft the manuscript, EP contributed to a global ischemia section; EM contributed with abstract and revised the manuscript for edits and important intellectual content. The manuscript has been read and approved by all the authors. The requirements for authorship have been met. Each author believes that the manuscript represents honest work.

#### Conflicts of interest

The authors declare that no conflict of interest exists.

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