Oxygen Therapy in Acute Ischemic Stroke - Experimental Efficacy and Molecular Mechanisms

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Abstract: Hyperbaric (HBO) or normobaric oxygen (NBO) therapy applied in acute ischemic stroke aims to increase oxygen supply to the ischemic tissue and to reduce the extent of irreversible tissue damage. Over the past decade, multiple studies have clarified the potential and limitations of oxygen therapy in preclinical stroke models. Considering that the reduction of the infarct size amounts to 30-40%, the cerebroprotection induced by HBO is moderate. In the experimental setting, the effective time window of HBO initiation is only a few hours. Higher pressures (2.5-3 ATA) are more effective. Even though oxygen therapy has some effectiveness in permanent cerebral ischemia without vascular recanalization, it appears more promising for bridging of a transient ischemic period until reperfusion of the penumbra takes place. Compared to HBO, the implementation of NBO to the clinical setting would be substantially less demanding. Although recent experimental NBO-studies are promising, significant effectiveness of NBO was only shown in transient cerebral ischemia and if started within a narrow time window of maximum 30 minutes. Some studies suggest that the effect of HBO is superior to NBO both during transient and permanent cerebral ischemia, even if treatment initiation is delayed. Limited experimental studies do not support an additive of a sequential combination of both therapies at present.

While the therapeutic potential of oxygen therapy in ischemic stroke was considerably better defined over the past years, the underlying cerebroprotective mechanisms of oxygen therapy remain to be fully elucidated. Recent studies have demonstrated that physical oxygen therapy indeed improves oxygen supply of the ischemic penumbra as well as the cellular bioenergetic metabolism. Therefore, the mitochondria including their role in apoptotic cell death pathways as well as the modification of the cellular hypoxia sensor HIF-1α are considered as potential ”downstream pathways” of oxygen therapy. Finally, its beneficial effects on the ischemic microcirculation suggest an important modification of various cell types within the neurovascular unit.

Keywords: Hyperbaric oxygen therapy, normobaric oxygen therapy, acute stroke, ischemic stroke, cerebral Ischemia, neuroprotection, efficacy, molecular mechanisms.

BACKGROUND

Stroke is the third leading cause of death and the most frequent cause of adult permanent disability in developed countries [1]. Brain ischemia, which arises from the occlusion of a cerebral artery due to thrombosis or embolism, accounts for about 80 percent of all strokes. Insufficient blood supply of parts of the brain causes immediate damage but also triggers delayed pathophysiological processes. The severity and the duration of the impairment of blood flow with the consecutive insufficient supply of oxygen to the brain tissue are primary determinants of brain damage [2, 3]. Because of the high energy demand of neurons and their limited capacity for energy storage, cellular hypoxia quickly leads to the breakdown of the oxidative mitochondrial metabolism and to anoxic cell death in the densely ischemic infarct core. In the less oligemic and initially viable peripheral ischemic zone, the so-called penumbra, various cascades can induce secondary tissue damage. Due to its already compromised blood supply, the penumbra is particularly vulnerable to additional hemodynamic and metabolic challenges. For example, peri-infarct depolarizations can induce secondary hypoxia and tissue damage in focal cerebral ischemia [3, 4].

On the cellular level, deleterious mechanisms include the neurotoxicity of excitatory neurotransmitters, the increased production of reactive oxygen species and the activation of inflammatory and apoptotic pathways. Because of their delayed onset, some of these processes are amenable to therapy and have hence received considerable attention during the last 15 years. Indeed, pharmaceutical modification of many of these targets in experimental studies improved outcome in animal models of stroke. Disappointingly, however, translation of these findings into the clinical setting has been unsuccessful in more than 130 phase 2 and 3 trials in ischemic stroke [5]. There are various reasons for this translational failure, but there is agreement among researchers that the potential of a stroke therapy ultimately depends on its power to intervene with key pathophysiological processes and that translation of experimental findings into clinical stroke management can only succeed if a consistent time window is used in experimental and clinical studies [6].

To date, early reopening of the occluded cerebral artery by recombinant tissue-plasminogen activator (rt-PA) is the only proven effective therapy in ischemic stroke [7]. One of the obvious consequences of early

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recanalization is that oxygen supply to the ischemic tissue is restored. However, only 5-10% of acute stroke patients currently receive rt-PA, mainly because of the limited time window for thrombolytic therapy. As alleviating ischemia-induced tissue hypoxia as early as possible is a pathophysiologically plausible albeit somewhat simplistic therapeutic strategy, various attempts have been made to raise tissue oxygen levels during ischemia. Physicochemical approaches that are based on the injection of substances with high binding capacity for oxygen such as perfluorocarbons, aqueous oxygen solutions or hemisynthetic hemoglobin were useful in some experimental ischemia studies [8-10] and combination therapy with these agents may be theoretically appealing. Nevertheless, the present review will focus on recent insights into the effectiveness and protective mechanisms of physical oxygen therapy in focal brain ischemia. Physical oxygen therapy takes advantage of the physiological principle that increasing inspiratory oxygen concentration and / or partial pressure results in a linear increase of oxygen partial pressure in the alveoli and the blood plasma. Inhalation of 100% oxygen can take place at ambient pressure, which is referred to as normobaric oxygen (NBO) therapy. Alternatively, in hyperbaric oxygen (HBO) therapy O_2 is inhaled under supraatmospheric pressure in pressurized chambers.

Herein, we review the basic principles of oxygen physiology in cerebral ischemia and critically summarize the available data regarding the efficacy of physical oxygen therapy in experimental and clinical stroke with an emphasis on essential variables for translation into stroke patient therapy. Although the protective mechanisms of oxygen therapy remain to be fully elucidated, recent insights into its cellualar effects will be presented.

PATHOPHYSIOLOGIC PRINCIPLES OF OXYGEN IN CEREBRAL ISCHEMIA

Understanding the regional variations of oxygen supply and demand and their underlying mechanisms is crucial with respect to the effect of oxygen therapy in both the healthy and especially the ischemic brain [11, 12]. Although it represents only 2% of the body's weight, the brain is supplied with about 15% of the cardiac output and consumes roughly 20% of the oxygen used by the whole body [13, 14]. This continuous demand of blood flow and oxygen supply is reflected by a high vulnerability to ischemia. In fact, brain tissue oxygen is depleted within seconds after complete interruption of blood flow [12, 15, 16]. On average, in man, global cerebral blood flow (CBF) is 40–60 mL/100 g/min and global cerebral metabolic rate for oxygen ($CMRO_2$) is 3–4 mL O_2/100 g/min [13, 17]. In thiopental-induced inhibition of synaptic transmission in non-human primates, about half of the oxygen consumed by the brain is linked to synaptic activity [18]. Fundamental neuronal and glial functions including intracellular enzyme reactions, biosynthesis of proteins, and the maintenance of the transmembrane ionic equilibrium (e.g. ATP dependent Na^+-K^+ pump) account for the other 50% of global $CMRO_2$ [11, 18, 19].

In the healthy organism the level of arterial blood oxygenation expressed as the oxygen partial pressure (PaO_2) is linearly related to that of the brain tissue (cerebral PtO_2). Under normoxic atmospheric conditions, the PaO_2 is around 100 mmHg and the cerebrovenous oxygen level ($PcvO_2$) is 35 to 40 mmHg [13]. Cerebral PtO_2 varies between 90 mmHg and much less than 35 mmHg mainly depending on the distance of the measuring probe from the capillary [14]. Furthermore, physiologic PtO_2 differs significantly between brain regions ranging from near 0 mmHg to arterial values as measured by surface and inserted electrodes [16, 20-23]. This heterogeneity correlates with the local capillary density and regional CBF which themselves correlate with neuronal density and ultimately with cellular metabolism [24-26].

Whereas the oxygen extraction fraction (OEF) in the healthy brain is approximately 40% for both gray and white matter [17], CBF as well as $CMRO_2$ is two to four times higher in gray matter than in white matter (≈60 versus 30 mL/100 g/min and 4 versus 1 mL/100 g/min, respectively) reflecting their different metabolic needs [27, 28]. Hence, the decline of PtO_2 after complete circulatory arrest is considerably slower in the white matter than in the rapidly metabolizing and oxygen consuming gray matter with its high capillary and cellular density [16].

Different thresholds of cerebral oligemia have been defined over the past decades [3, 29, 30]. Complete energy failure with break down of the cellular ion equilibrium, anoxic depolarization and consecutive necrotic infarction manifest with CBF values of less than 12 mL/100 g/min in the ischemic core of focal cerebral ischemia [31]. However already the decline of cortical CBF just below normal levels is associated with alterations of gene expression and partial inhibition of protein synthesis [32]. Mean CBF values below 23 mL/100 g/min provoked EEG slowing, and EEG flattening occurred if CBF fell below 15 ml/100 g/min [33, 34]. Excitatory neurotransmitters are released at a residual CBF of about 18 mL/100 g/min [35]. At this blood flow threshold oxygen supply becomes insufficient resulting in depletion of high energy phosphates such as phosphocreatine and ATP, accumulation of lactic acid with decreased brain tissue pH, and membrane depolarization [3]. In addition, reduced heat conduction in the malperfused penumbra can induce local hyperthermia (2-3 °C) triggering a vicious circle with further progression of ischemia / hypoxia [36, 37].

When breathing room air (21% oxygen) at 1 atmosphere absolute (1 ATA), about 97% of the oxygen transported in the blood is chemically bound to hemoglobin (Hb) and only 3% is physically dissolved in the blood plasma [14]. Considering an average hemoglobin concentration of 14 g/100 mL blood, the oxygen binding capacity of hemoglobin of 1.39 mL O_2/ g Hb at 37 ºC, and a physiological arterial Hb saturation of 97% (SaO_2), the total volume of oxygen bound to hemoglo-

bin for every 100 mL of blood is about 18.9 mL. Under these circumstances, only 0.3 mL O_2 are physically dissolved per 100 mL of blood [14]. Consequently, only 2-3% of the cerebral metabolic rate for oxygen ($CMRO_2$) are covered by the dissolved oxygen fraction under normoxic conditions [38].

Diffusion of oxygen along the pressure gradient is the driving force behind oxygen transport from the air in the alveoli to the blood and from there to the tissue and the mitochondria [14, 39]. When breathing 100% instead of 21% oxygen at 1 ATA (NBO) or 100% oxygen at even higher ambient pressure (HBO) not only arterial oxygen pressure (PaO_2) but also brain tissue oxygen levels (PtO_2) increase [40-45]. For a pressure of up to 6 ATA and a constant CBF a linear relationship (6:1) was shown between the inspired oxygen pressure (PiO_2) and cerebral PtO_2 [46].

As hemoglobin is already nearly saturated (SaO_2 ~97%) under normoxic atmospheric conditions (21% O_2 at 1 ATA), normobaric or hyperbaric oxygenation can hardly increase the amount of oxygen bound to hemoglobin. Instead, the proportion of physically dissolved oxygen becomes more relevant. Given that the Bunsen solubility coefficient of oxygen in blood (37 ºC) is 0.003 mL O_2/ mmHg/ mL blood, increasing PaO_2 from 100 mmHg to 663 mmHg (100% O_2 at 1 ATA) raises the physically dissolved oxygen fraction from 0.3 mL to about 2 mL per 100 mL of blood. When breathing 100% oxygen at 2.5 ATA 5.4 mL O_2 are physically dissolved in 100 mL of blood [47]. Taking into account that the normal arteriovenous oxygen difference at rest is between 4-6 mL per 100 mL blood, this is sufficient to meet the baseline O_2 demand of the human brain and the whole body. Indeed, under experimental hyperbaric conditions even life without any hemoglobin is possible as demonstrated in pigs surviving a complete blood-plasma-exchange [48].

Based on these physiological principles, it has been hypothesized for decades that the surplus of physically dissolved oxygen by HBO or NBO may provide suffient oxygen to hypoxic-ischemic brain areas. Increasing the dissolved oxygen plasma fraction by oxygen therapy may be particularly relevant in focal cerebral ischemia [49], because capillary plasma flow was maintained in spite of a substantial reduction of cortical CBF [50]. However, evidence backing up this hypothesis has been missing until recently (see below).

EVIDENCE FROM PRECLINICAL STUDIES FOR EFFICACY OF OXYGEN THERAPY IN STROKE

Multiple factors can be expected to affect the therapeutic potential of oxygen therapy. As clinical ischemic stroke is very heterogeneous, these factors include severity and extent of cerebral hypoperfusion as well as duration and persistence of vascular occlusion. Therapeutic variables encompass the time window (i.e. interval between symptom-onset and initiation of therapy), as well as the duration, frequency (single versus repeated) and pressure of oxygen exposure.

Since the early 1960s, numerous experimental studies focusing on HBO in focal and global cerebral ischemia have been carried out [51-55]. The majority of these studies found HBO to be cerebroprotective. However, an important shortcoming of most studies published until the late 1990s was, that important validity criteria for experimental outcome studies were not fulfilled: For example, physiologic monitoring was not performed, the animal species and the experimental model were unreliable, and outcome parameters were inappropriate. Furthermore systematic dose-finding studies were missing just as studies determining the therapeutic time window or comparing models of transient versus permanent ischemia [49].

In the past decade HBO has relived a scientific renaissance [56, 57]. Moreover, the therapeutic potential of NBO with the advantage of its minimal technical and logistical demands has been recognized [54, 57-64]. Sophisticated diagnostic developments, especially in the field of neuroimaging, promote this trend [60, 63, 65-68]. The failure of translational research in the stroke arena has prompted a reevaluation of preclinical study designs including some suggestions for assessment of validity [69]. Accordingly, the following critical review of experimental outcome studies using oxygen therapy (Table **1** and **2**) includes scores assessing the quality of individual studies (Table **3**), which have been developed to improve the validity of preclinical studies (Table **4**) [6, 70].

Oxygen Therapy in Transient Ischemia

With exception of one study showing no functional benefit [71], all other animal studies using HBO treatment in *transient* experimental focal cerebral ischemia consistently reported beneficial effects on infarct size and / or neurobehavioral deficits regardless of whether HBO was initiated before onset of [72, 73] or during ischemia [49, 67, 68, 73-79] or within 6 h of reperfusion [74, 80-86] (Table **1**). In contrast, when initiated after 12 h of reperfusion, HBO aggravated the ischemic injury [80, 83]. Interestingly, one study showed beneficial effects for repetitive HBO (2.5 ATA) even when started as late as 24 hours after transient MCAO (tMCAO) [86].

There are limited data regarding the optimal pressure of HBO. The majority of investigators reporting a beneficial effect of HBO applied pressures greater than 2.5 ATA [49, 72, 79, 82, 85, 86], mostly 3 ATA [67, 68, 73, 74, 76, 80, 81, 83, 84], whereas Roos *et al*. [71] and Hou *et al*. [75] used 2 ATA. However, HBO 1.5 ATA was more effective than 100% oxygen (NBO) or room air at 1 ATA in a cat model of focal ischemia also [77, 78]. Only two studies directly compared the effectiveness of HBO at different pressures: Veltkamp and colleagues found that rats receiving intra-ischemic HBO at 2.5 ATA had better behavioral scores and smaller infarct volumes than NBO whereas 1.5 ATA had no effect in rats [49]. More recently, an extensive study by Eschenfelder and collaborators showed that infarct size and postischemic functional deficit were inversely correlated with pressure. Only pressures ≥2.5

Table 1. Experimental Studies of Hyperbaric Oxygen Treatment in Focal Cerebral Ischemia

Name Year	Animal Model	Time of ischemia	Treatment latency	Treatment	Treatment duration	Outcome	Quality Score*
Corkill 1985	Gerbil permanent CCA ligation	P	<1 h	100% O_2 2 ATA or 1.5 ATA vs. air	1× 1 h or 1× 30 min	Videodensitometry: interhemispheric difference↑	2
Weinstein 1986	Gerbil transient CCA ligation	20 min	0 min	100% O_2 1.5 ATA vs. air	1× 15min	Survival↑, infarct↓, neuroscore↑	2
Burt 1987	Gerbil permanent CCA ligation	P	<30 min	100% O_2 1.5 ATA vs. air	1× 36 h 1x 18 h on/of 36h	Survival↑, infarct↓	1
Weinstein 1987	Cat transient vesselocclude	6 h or 24 h	1, 2, 3, 6 h 0, 2, 24 h	100% O_2 1.5 ATA vs. air	1× 40min	0, 1, or 2 h: Infarct↓, Neuroscore↑ 3, 6 or 24 h: no diff.	1
Kawamura 1990	Rat transient filament	4 h	0.5-1.5 h or 2.5- 3.5 h	100% O_2 2 ATA vs. air	1× 30min	Infarct↓, edema↓ (only in 2.5-3.5 h group) In 0.5-1.5 group: no diff.	3
Reitan 1990	Gerbil permanent ICA	P	40 min	100% O_2 1,875 bar vs. air	1× 2 h, or 1× 4 h	Survival↑ (no other outcome parameter)	6
Roos 1998	Rat transient filament	variable	3-90 min	100% O_2 2 ATA vs. air	1× 30 min 5× 30 min	No functional benefit (no histology)	3
Atochin 2000	Rat transient filament	2 h	before	100% O_2 2.8 ATA vs. air	1× 45min	Infarct↓, neuroscore↑, neutrophil accumulation↓	1
Chang 2000	Rat transient filament	1 h	0 min or 60 min	100% O_2 3 ATA vs air 3 ATA	2× 1.5 h	Infarct↓, neuroscore↑, intermediate effect of hyperbaric air	4
Sumani 2000	Rat permanent coagulation	P	10 min	100% O_2 3 ATA vs. air	1× 2 h	Infarct↓, no change in lipid peroxidation	4
Veltkamp 2000	Rat transient filament	75 min	12 min	NBO 1.5 ata, 2.5 ATA vs. air	1× 1 h	Infarct at 2.5ata↓, neuroscore, no effect of 1.5 ata	7
Badr 2001	Rat transient filament	2 h	6 h	100% O_2 3 ATA vs. air	1× 1 h	Infarct↓, glutamate↓, glucose↓, pyruvate↓	1
Hjelde 2002	Rat permanent filament	P	10 min	100% O_2 2 ATA vs. air	1× 230 min	MRI: DWI: no change Neutrophil infiltration: no change	2
Yang 2002	Rat transient filament	1 h	0 min	100% O_2 2.8 ATA vs. air	1× 1 h	Edema and neuronal shrinkage↓, dopamine increase↓	2
Yin 2002	Rat transient filament	2 h	6 h	100% O_2 3 ATA vs. air	1× 1 h	Infarcts↓, Increase in COX-2↓	1
Miljkovic-Lolic 2003	Rat transient coagulation	60 min MCA	before or 0 min	100% O_2 3 ATA vs. air	1× 1 h	Infarct↓, Neuroscore↑ leukocyte infiltration↓	5

(Table 1). Contd…..

Name Year	Animal Model	Time of ischemia	Treatment latency	Treatment	Treatment duration	Outcome	Quality Score*
Yin 2003	Rat transient filament	2 h MCA	8 h	100% O_2 2.5 ATA vs. air	1× 2 h	Neuroscore↑ Apoptotic bodies↓ DNA fragmentation↓	4
Lou 2004	Rat transient and permanent filament	90 min P	3, 6, 12 h	100% O_2 3 ATA vs. air	1× 1 h	Infarct↓ Neuroscore↑ (3, 6 h), Infarct↑, Neuroscore↓ (12 h) Infarct and Neurscore not different in perm. ischemia.	4
Schabitz 2004	Rat permanent filament	P	2 h	100% O_2 2 ATA vs. air	1× 1 h	MRI: DWI and T2-lesion↓ Neuroscore↑ Lipid peroxid.: no difference	5
Veltkamp 2005a	Rat transient filament	120 min	40 min	100% O_2 3 ATA vs. air	1× 1 h	Infarct↓ MRI: DWI and T2w lesion↓	4
Veltkamp 2005b	Rat and mouse transient filament	2 h	40 min	100% O_2 3 ATA vs. air	1× 1 h	MR: T2w lesion↓ BBB permeability↓	5
Henninger 2006	Rat permanent thrombembolic	P	3 h	100% O_2 2.5 ATA vs. air	1× 1 h	Infarct↓ MRI- ADC and T2w lesion↓	4
Veltkamp 2006b	Rat, Mouse permanent/ transient Coagulation/ filament	P/ 120 min	45 min or 120 min	100% O_2 3 ATA, NBO, NBO plus 100% O_2 3 ATA vs. air	1× 75 min or 7× 75 min	MCA-Coagulation: Infarct↓, TUNEL neurons↓ Perm. filament: no effect Repeated HBO: No add. effect	5

*see Table **3** and **4** for details of quality criteria and scores.

Table 2. Animals Studies of Normobaric Oxygen Treatment in Focal Cerebral Ischemia**

Name Year	Animal Modell	Time of ischemia	Treatment initiation	Treatment	Treatment duration	Outcome	Quality Score*
Miamoto and Auer 2001	Rat transient filament	80 min	0 min	100% O_2 1 ATA	80 min	Necrosis↓	2
Flynn and Auer 2002	Rat transient filament	1 h	Pre-, intra- and postischemic	100% O_2 1 ATA		Behavioural function↑, smaller infarct size↓, continuous therapy offers greatest benefit.	5
Singhal 2002a	Rat transient filament	2 h	42 min 15 min 30 min 45 min	100% O_2 1 ATA	1× 78 min 120 min 105 min 90 min	DWI and T2 image↓, infarct volume↓	2
Singhal 2002b	Rat transient filament	2 h	0 min	100% O_2 1 ATA	1× 3 h	Infarkt volume↓, no increase of oxidative stress	2
Kim 2005	Rat transient filament	1-4 h	5 min	100% O_2 1 ATA	1-4 h	Functions↑, infarct size↓, no changes in superoxide and MMP	4

(Table 2). Contd…..

Name Year	Animal Modell	Time of ischemia	Treatment initiation	Treatment	Treatment duration	Outcome	Quality Score*
Liu 2006	Rat transient filament	90 min	0 min or 90 min	70% O_2 95% O_2 100%O_2 1 ATA	1× 90 min	Infarct volume↓, neurologic function↑, ROS↓, MMP-9 expression↓ and caspase-8 cleavage↓ in the penumbra	6
Veltkamp 2006b	Rat permanent filament and Mouse permanent filament/ coagulation		45 min	100% O_2 1 ATA	1× 75 min	Infarct volume↓, apoptosis↓ in transient filament model and coagulation model. No effect in permanent filament model	5
Heninger 2007	Rat permanent transient filament		30 min	100% O_2 1 ATA	1× 6h or 1× 3h	Infarct volume↓, preservartion of perfusion/diffusion mismatch, apoptosis↓	5

*see Table **3** and **4** for details.
**modificated by Helms *et al.* 2005.

Table 3. Summary of Validity Scores for Preclinical Outcome Studies Apply HBO or NBO in Experimental Focal Ischemia

Name Year	1	2	3	4	5	6	7	8	9	10	Score
Corkill 1985					*	*					2
Weinstein 1986						*				*	2
Burt 1987										*	1
Weinstein 1987						*					1
Kawamura 1990	*			*		*					3
Reitan 1990	*	*			*	*	*			*	6
Roos 1998	*		*		*						3
Atochin 2000						*					1
Chang 2000	*	*	*			*					4
Sumani 2000	*			*		*		*			4
Veltkamp 2000	*		*	*	*	*	*			*	7
Badr 2001						*					1
Hjelde 2002	*			*							2
Yang 2002	*					*					2
Yin 2002						*					1
Miljkovic-Lolic 2003	*		*	*		*				*	5
Yin 2003	*			*		*	*				4
Lou 2004	*			*		*	*				4
Schaebitz 2004	*		*	*		*	*				5
Veltkamp 2005a	*			*	*	*					4
Veltkamp 2005b	*			*	*	*	*				5
Henninger 2006	*	*		*			*				4
Veltkamp 2006a	*			*		*					3

(Table 3). Contd…..

Name Year	1	2	3	4	5	6	7	8	9	10	Score
Veltkamp 2006b	*			*	*	*	*				5
Muyamoto and Auer 2000				*		*					2
Flynn and Auer 2002	*			*	*	*	*				5
Singhal 2002a				*		*					2
Singhal 2002b				*		*					2
Kim 2005	*			*		*				*	4
Liu 2006	*			*	*	*	*			*	6
Veltkamp 2006b	*			*	*	*	*				5
Heninger 2007	*			*	*	*				*	5

ATA resulted in a statistically significant protective effect of HBO [82]. However, the latter findings cannot be directly translated into treatment during ischemia because oxygen therapy was started after reperfusion. Up to now there are neither experimental findings defining the optimal duration for oxygen therapy nor data exist showing potential limitations of higher oxygen pressures with regard to oxygen toxicity in transient brain ischemia.

Table 4. Quality Criteria for Preclinical Studies in Focal Ischemia (Modified from Macleod *et al.* 2005; Dirnagl, 2006)

1. Random allocation to treatment or control
2. Blinded induction of ischemia
3. Blinded assessment of outcomes
4. Monitoring of physiologic parameters
5. The dose / response relationship was investigated
6. Assessment of at least two outcomes
7. Time of outcome assessment in chronic phase (5 to 30 days)
8. Appropriate animal model (aged, diabetic, hypertensive)
9. Sample size calculation
10. Mortality reporting

Effectiveness of NBO in transient experimental ischemia was initially suggested by the pioneering studies of Auer´s group [58, 59] (Table **2**). For NBO in experimental tMCAO, infarct volumes were inversely proportional to the interval between onset of ischemia and treatment [60]. Compared to normoxic controls, infarction volumes were significantly attenuated when NBO was initiated within 30 mins after onset of ischemia [60, 61, 65, 87, 88]. Concerning NBO during reperfusion only, results differ. Whereas Liu *et al*. failed to show a structural or behavioral benefit from NBO during reperfusion [88], Flynn and Auer reported equal benefit for NBO during tMCAO or reperfusion [58]. Furthermore, augmented benefit was observed for continuous intra-ischemic and reperfusion eubaric hyperoxemia [58]. In contrast to these promising results, in our experiments, early prolonged NBO failed to reduce infarct volume, whereas delayed HBO (3 ATA) did show neuroprotection [89]. Combination of early NBO and delayed HBO had no additional effect [89]. Similarly, Hou *et al*. reported that HBO (2 ATA) but not NBO started during ischemia promoted neuroprotection [75].

Oxygen Therapy and Permanent Ischemia

As early spontaneous reperfusion occurs only in the minority of stroke patients [90], experiments testing oxygen therapy in permanent ischemia are particularly relevant for translational outcome studies. The data regarding efficacy of oxygen therapy in permanent ischemia are controversial (Table **1** and **2**). In three studies, HBO (2 ATA) [66, 91] and prolonged NBO (for 6 h) [65], respectively, reduced infarct size after filament-induced permanent MCAO (pMCAO). Shorter NBO treatment (for 3 h) was not beneficial in the latter study [65]. In contrast, we found that in rodent pMCAO models, the efficacy of oxygen therapy was linked to the volume of the ischemic tissue. NBO and significantly more HBO (3 ATA) initiated within 120 mins reduced infarct size after coagulation of the MCAO distal of the lenticulostriate branches, which results in cortical infarcts. In contrast, oxygen therapy failed to improve outcome after filament-induced pMCAO in rats and mice, which causes extensive cortical and subcortical infarcts [64]. This is consistent with findings from other groups in which HBO was protective after permanent ligation or electrocoagulation of the MCA [44, 92], but failed to show a benefit of HBO after filament pMCAO [83, 93].

Concerning the time window in coagulatory pMCAO, HBO was effective when initiated within 1-3 h but not after 6 h [64, 92]. After filament pMCAO, however, failure of HBO was observed even when started as early as 10 min [93], 45 min [64] or 3 h [83], although Schäbitz and colleagues found a benefit of HBO when initiated within 2 h [66]. Interestingly, prolonged NBO was protective when initiated within 30 min only [65].

Combination of NBO and HBO (3 ATA) was more efficient than NBO alone [64]. Neither repetitive HBO treatments on subsequent days [64] nor repetitive HBO

during the first 24 h [92] provided additional protection over a single administration in permanent ischemia.

EFFICACY OF OXYGEN THERAPY IN CLINICAL ISCHEMIC STROKE

Although hundreds of stroke patients have been treated with HBO or NBO over the last 40 years, the usefulness of oxygen therapy in ischemic stroke has remained an unresolved issue [53, 94]. The earlier studies were case reports or case series lacking a prospective standardized, controlled clinical trial design [95-101]. Neuroimaging studies differentiating between ischemic and hemorrhagic stroke were not performed and outcome assessment was largely inadequate. The results of these studies were mainly positive but inclusion criteria were heterogenuous, and evaluation of putative benefit lacked the comparison with control patients and the objectivity of blinded outcome assessment. From a present perspective, these reports can be summarized as interesting observations suggesting feasibility of administration in acute stroke and some unsystematic data regarding safety.

Subsequently, three small randomized controlled clinical trials were performed none of which showed efficacy of HBO in ischemic stroke [102-104]. Due to the inadequate sample size, a systematic review concluded that evidence from these three trials is insufficient to provide clear guidelines for practice [94]. Other shortcomings of these trials included a very long time window. Therapy was delayed up to 7 days after symptom onset [102], with most patients treated 12 hours or more after stroke [103, 104]. Only one study used brain imaging to exclude hemorrhage [104]. Anderson *et al*. treated controls with hyperbaric air [102] and Rusyniak *et al*. with NBO [104], although, both treatments are not part of the standard management of ischemic stroke, and may themselves improve outcome based on preclinical data [58, 60, 61, 65, 74, 87].

Considering its ease of administration, ubiquitous availability and inexpensiveness, it is surprising, that so far only two human studies evaluating NBO for stroke treatment have been performed [62, 105]. Ronning and Guldvog conducted a large prospective quasi-randomized study [105], in which 550 patients within 24 hours after onset of a stroke were prospectively enrolled and allocated to either treatment with supplemental oxygen at a rate of 3 liters per minute *via* a nasal probe or normobaric air for 24 hours. As the one-year survival was significantly higher among controls with Scandinavian Stroke Scale (SSS) scores <40, the authors suggested that supplemental oxygen should not routinely be given to stroke patients with minor or moderate strokes. However, given that breathing oxygen administered through a nasal catheter at a rate of 3 liters per minute has virtually no effect on blood oxygen levels compared to room air [106], the efficacy of NBO cannot be appreciated on the basis of this study. More recently, Singhal and coworkers investigated the effects of NBO delivering 45 liters O_2 per minute *via* a facemask in acute ischemic stroke [62]. 16 patients within 12 hours after stroke onset and with a PWI / DWI mismatch on MRI were randomized to 8 hours of NBO or room air. NIH Stroke Scale Scores (NIHSSS) significantly improved at 24 hours, tended to improve already during NBO at 4 hours and 1 week after NBO, but no significant difference was found 3 months later [62]. DWI lesion volumes were significantly reduced by NBO at 4 hours, but not at subsequent time points. Independent from arterial recanalization cerebral blood volume (CBV) and blood flow (CBF) within ischemic regions improved significantly at 4 and 24 hours in NBO-treated patients. The authors noted an increased incidence of asymptomatic hemorrhagic transformation in hyperoxia-treated patients (50% versus 17%) emphasizing the need for further evaluation of safety of NBO [62]. A larger NIH-funded phase II study initiated by the same investigators is ongoing [NCT00414726].

Although previous clinical studies using HBO or NBO for acute ischemic stroke show some promising results, lacking congruence of preclinical and clinical study designs is a major limitation for translation. Critical variables such as time window and dose cannot be directly adapted from rodent ischemia models to human stroke and potential additive effect or at least compatibility with thrombolytic therapy have to be explored. While most preclinical studies suggest a superior effectiveness of HBO compared to NBO, this remains to be shown in the clinical setting. Sequential combination of both oxygen therapy modalities may be useful.

CEREBROPROTECTIVE MECHANISMS OF OXYGEN THERAPY

Oxygen Delivery

The cellular and molecular mechanisms of cerebroprotection by oxygen therapy are only partially understood at present (Fig. (**1**)). Severe ischemia causes rapid depletion of brain energy stores. Neuronal energy production depends almost exclusively on oxidative phosphorylation in the mitochondria [2], which is aborted during severe hypoxia. As the critical oxygen tension required for mitochondrial function is very low (1.5 mmHg) [14, 107, 108], improving the oxygen delivery to ischemic-hypoxic tissue appears to be a simple and plausible therapeutic concept to shift the ischemic threshold for cell death. Remarkably, the experimental evidence supporting this basic therapeutic concept of physical oxygen therapy had not been available until recently.

Utilizing laser-Doppler flowmetry, Sunami *et al*. showed that - at constant $PaCO_2$ - hyperbaric oxygen (3 ATA) did not further affect CBF reduction seen in the ischemic periperhy during experimental focal cerebral ischemia. At the same time HBO significantly raised the arterial oxygen content. The calculated increase in the oxygen supply to the ischemic periphery *via* HBO was quoted to be 20% [44].

NBO significantly raised penumbral PtO_2 in rats measured by *in vivo* electron paramagnetic resonance

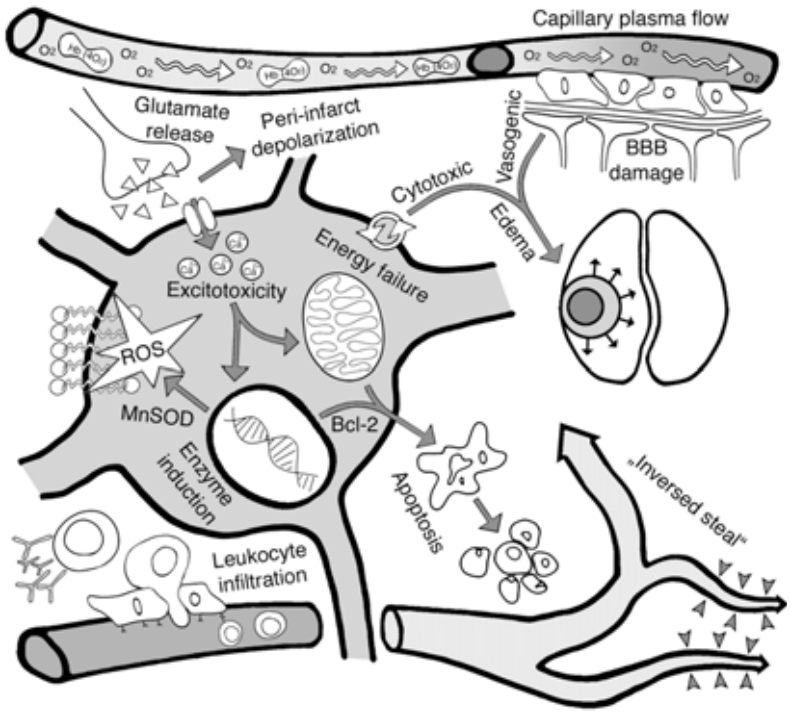

Fig. (1). Simplified overview of potential neuroprotective mechanisms of oxygen therapy in ischemic stroke including increased oxygen delivery, reduced excitotoxicity and peri-infarct depolarization, improved energy metabolism, induction of apoptotic inhibitors and free radical scavengers, preservation of the blood-brain barrier, reduced brain edema and inhibition of leukocyte infiltration.

oximetry (EPRO) [109]. Indeed, the same investigators showed that 95% normobaric oxygen during ischemia was able to maintain penumbral PtO_2 close to pre-ischemic values [88]. Using laser speckle photometry, Shin *et al.* recently reported that postischemic NBO increased the oxyhemoglobin concentration and cerebral blood flow, and resulted in a reduction of peri-infarct depolarization in ischemic core and penumbra [110]. Furthermore, Takano showed that also the duration of the peri-infarct depolarization-induced PtO_2 decline was shortened by NBO [111]. In contrast, when using the injected nitroimidazole EF-5 as an extrinsic hypoxia marker, our group found a reduction of the extent of the hypoxic area after MCAO only in HBO (3 ATA) but not in NBO treated mice [112]. Similarly, the intrinsic cellular hypoxia sensor hypoxia inducible factor HIF-1α was only reduced in HBO treated mice after MCAO [112]. In conclusion, HBO improves tissue oxygen supply in the oligemic penumbra.

Effects on Cerebrovascular Autoregulation

In the nonischemic brain, hyperoxia leads to vasoconstriction and a consecutive decrease in CBF [46, 113-120]. Given its important role in cerebrovascular autoregulation [121], nitric oxide (NO) was the focus of several oxygen therapy-studies [113, 114, 120, 122]. Reactive oxygen species (ROS)-conditioned inactivation [114, 120] of endothelial nitric oxide synthase isoform (eNOS)-derived NO [113] was most likely the underlying mechanism of hyperoxia-mediated vasoconstriction. Consequently, oxygen therapy and particularly HBO have been regarded as potentially harmful in ischemic stroke because they may further decrease CBF in oligemic areas. However, the normal cerebrovascular regulation is profoundly altered by ischemia or even lost in ischemic regions [123, 124]. It has been speculated that oxygen therapy causes an "inverse steal" favoring the blood supply of ischemic regions [53, 125], but the actual data supporting this concept are sparse. Recent rodent [60] and human [62] studies using MRI observed an increased relative cerebral blood volume (CBV) within initially hyoperfused areas after therapy with NBO. Further application of *in vivo* neuroimaging methods should be able clarify this issue.

Effects on Ischemic Energy Metabolism

Experimental studies using *in vivo* MRI suggest that oxygen therapy-mediated protection of brain tissue begins at a very early phase of ischemia [60, 65, 67]. This timing is compatible with a partial prevention of secondary bioenergetic failure in the penumbra. In models of *in vitro* ischemia and *in vitro* hypoxia, attenuation of cellular damage by HBO and NBO was paralleled by a restitution of purine nucleotide levels (ATP/ADP and GTP/GDP ratios) [126, 127]. Following global cerebral ischemia due to bilateral carotid artery ligation in rats HBO administered as late as 3 hours after brain ischemia prevented further increase in cerebral lactate, tended to increase cerebral ATP levels and produced a significant increase in survival time [128]. This may correspond with measurements of lactate and pyruvate in

the cerebrospinal fluid of HBO-treated stroke patients [129] and in striatal microdialysates of HBO-treated rats [81]. Using ATP bioluminescence imaging, we could recently show that the area of ATP depletion after focal ischemia is significantly reduced in animals treated with either HBO at 3 ATA and NBO compared to air (unpublished data). Finally, in a recent human magnetic resonance spectroscopy (MRS) study, NBO was found to reduce brain lactate and preserve N-acetyl-aspartate (NAA) within regions of ischemia, again suggesting improvement of oxidative metabolism [63].

Effects on Excitotoxicity and Free Oxygen Radicals

Ischemic brain injury results from a complex sequence of pathophysiological events. Besides failure of the aerobic energy metabolism, one of the major pathogenic mechanisms of this cascade is excitotoxicity caused by an excessive release of excitatory amino acids into the extracellular space [2]. After transient MCAO in rats, striatal glutamate concentrations measured by microdialysis decreased almost to preischemic levels in HBO treated rats [81]. In another rodent microdialysis study HBO attenuated the excessive release of striatal dopamine during ischemia / reperfusion and reduced histological tissue damage compared to the control rats [79]. Considering the abundance of corticostriatal glutamatergic and nigrostriatal dopaminergic innervation and taking into account that the striatum is particularly susceptible to ischemia, the HBO-mediated attenuation of the release of excitatory amino acids during cerebral ischemia might contribute to the neuroprotective effect of HBO. However, it is currently unclear whether this reflects a specific therapeutic effect or an epiphenomenon of HBO-induced protection.

In various experimental ischemia models free oxygen radicals have been shown to play a considerable role in brain damage [130, 131]. After cerebral ischemia, reactive oxygen species (ROS) react with cellular macromolecules including DNA repair enzymes, transcription factors and other proteins participating in apoptotic signaling pathways [130, 131]. In ischemic stroke when the components of the respiratory chain are reduced and molecular oxygen is present, ROS generated by brain mitochondrial electron transport may escape endogenous antioxidant defenses and promote highly damaging hydroxyl radical activity [132]. As oxygen therapy raises oxygen availability, the enhanced formation of toxic free radicals is regarded as a potentially dangerous adverse effect of oxygen therapy. Indeed some studies showed increased blood and brain tissue levels of free radicals after HBO treatment in healthy humans [133] and mammals [134-136], respectively. Neurotoxic effects of HBO that are mainly mediated by the generation of ROS usually occur at pressures exceeding 4 ATA and after extended periods of administration [137, 138]. As measuremnt of ROS *in vivo* is difficult, surrogate parameters include measurement of downstream products such as lipid peroxidates or markers of radical-induced damage such as blood-brain barrier damage.

Several studies on brain ischemia using HBO at therapeutic pressures between 2 and 3 ATA found no damaging effects of free radical reactions *via* lipid peroxidation [44, 52, 66, 93, 139]. HBO treatment did not increase staining of 4-hydroxy-2-nonenal (HNE)-modified proteins and pattern of c-Fos induction, two markers of oxidative stress [66]. Although HBO-induced production of free radicals was observed in a rabbit model of global cerebral ischemia it was not linked to an increase in lipid peroxidation [139]. Pharmacological treatment with a radical spin trap agent had no surplus protective effect when compared to HBO treatment alone [140]. As free radical have also positive effects under certain circumstances, it has been speculated that enhanced production of superoxide and hydrogen peroxide radicals in HBO treated animals may be beneficial in cerebral ischemia [141]. However, superoxide ion concentration measured by hydroethidine fluorescence was lower in the ischemic hemisphere of HBO treated than in air breathing animals in one of our recent studies (unpublished data).

Similar to HBO, NBO did not augment markers of oxidative stress after ischemia [61, 87, 88]. After transient focal cerebral ischemia levels of heme oxygenase-1 (HO-1), a heat shock protein induced by oxidative stress, and protein carbonyl formation did not differ between NBO and normoxic controls [61]. Cellular markers of free radical generation, e.g. hydroethidine or 8-hydroxy-20-deoxyguanine, and other indirect markers of oxidative stress such as matrix metalloproteinase (MMP)-2, MMP-9 and caspase-8, were either unchanged or even decreased after NBO in experimental ischemia [87, 88]. In conclusion, there is no convincing evidence that oxygen therapy increases free oxygen radical production and related damage.

Inflammatory Processes

Cerebral ischemia triggers a complex interaction of systemic and local cellular and humoral inflammatory cascades [142, 143]. On the one hand, inflammatory mechanisms are involved in cell damage and repair after ischemia. For example, upregulation of pro-inflammatory cytokines and chemokines leads to the expression of endothelial-leukocyte adhesion molecules, facilitating migration of neutrophils, monocytes and lymphocytes across the blood-brain barrier. In animal models of sepsis or systemic inflammatory response syndrome, HBO attenuated important inflammatory processes including cytokine release, regulation of adhesion molecules and leucocyte migration [144-146]: Interestingly, this anti-inflammatory effect may participate in protection against ischemic damage. In experimental stroke, protective effects of HBO were associated with the inhibition of neutrophil sequestration [72, 73]. *In vitro* studies suggest that HBO treatment downregulates beta-2-integrin expression on leucocytes [147] and inhibits intercellular adhesion molecule-1 expression on endothelial cells [148]. HBO also reduced the post-ischemic expression of cyclooxygenase-2 (COX-2), a key enzyme for prostanoid syn-

thesis, in one study [84]. Prostanoids are deleterious in *in vitro* studies of excitotoxicity and oxygen–glucose deprivation, and COX-2 enzymatic reaction produces superoxide radicals, which have deleterious effects in cerebral ischemia [149]. However, the findings regarding the effects of oxygen therapy on inflammatory mechanisms remain fragmentary at present.

Effects on the Ischemic Microcirculation

Although it has been recognized for some time that focal cerebral ischemia does not selectively injure individual cell types such as neurons but that the microcirculation is the trigger and target of damage in cerebral ischemia [150], neuroprotection was the predominant objective of cerebrovascular research in the 1990s. Compared to this neurocentric view of cell death, the concept of a "neurovascular unit" emphasizes the complex structural and functional interdependency of different cell types that is affected in focal cerebral ischemia [151]. Ischemia-induced disruption of the blood-brain barrier (BBB), which is formed by the endothelium, the extracellular matrix and the astrocytes, is the central part of important complications of ischemia, vasogenic edema and secondary hemorrhage [152, 153]. In acute stroke patients receiving thrombolysis MRI correlates of BBB breakdown preceded hemorrhagic transformation [154, 155].

A number of studies suggest that oxygen therapy may reduce postischemic micocirculatory damage. Mink and Dutka found a reduction of BBB leakage early after global cerebral ischemia [139]. More relevantly, HBO therapy during transient focal cerebral ischemia reduced early and delayed BBB damage in rats and mice as measured by post-contrast T1-weighted MRI and diminished extravasation of Na-fluorescein, respectively [68]. Similar to the infarct volume reduction, the vasogenic edema assessed on T2-weighted MR-images and histological sections was significantly lower in HBO-treated rats in that study [68]. Qin *et al*. showed that secondary hemorrhagic transformation was less pronounced after transient filament-induced MCAO in HBO treated rats [156]. Similar protective effects on the postischemic BBB have been reported for NBO [61, 65].

In a recent unpublished study, we also found a reduction of postischemic BBB damage and of postischemic hemorrhagic transformation both by NBO and by HBO in a thromboembolic MCAO model with thrombolysis. The molecular mechanisms underlying these effects are largely unknown. HBO attenuated the postischemic degradation of the basal laminar component laminin-5 in one study [76]. Furthermore, postischemic upregulation of matrix-metalloproteinase-9 (MMP-9), a key enzyme of basal lamina degradation, was blocked by either NBO [87] or HBO [76]. Although these effects probably are not primary but rather downstream effects of oxygen therapy, they suggest a potential for oxygen therapy as a protector of the "neurovascular unit" which may be particularly relevant in the setting of thrombolytic therapy.

Effects on Apoptosis and the HIF pathways

Besides cell death due to bioenergetic failure, excessive calcium influx, reactive oxygen species, mitochondrial and DNA damage trigger delayed programmed cell death pathways, which contribute substantially to the ischemic tissue damage in the penumbra [131, 157]. In neonatal hypoxia-ischemia as well as focal and global cerebral ischemia models in rodents, markers of apoptotic cell death were decreased after HBO treatment [64, 85, 158-160]. This includes a reduction in the number of TUNEL (terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling) staining cells, a marker for apoptotic DNA fragmentation [64, 85], a reduced expression and activity of neuronal caspase-9 and caspase-3 [85, 158, 159] and an attenuation of poly-ADP-ribose polymerase cleavage [158], a nuclear polymerase mediating DNA-repair. Similarly, NBO reduced selective neuronal death in the hypoperfused tissue [65]. Interestingly, Henninger *et al*. found an increase of necrotic as well as caspase-3-mediated apoptotic cell death in the otherwise unaffected non-ischemic hemisphere of NBO-treated animals relative to air-treated controls [65]. Although the mitochondria as key organelles in programmed cell death are a plausible candidate target of oxygen therapy, there are no data to support this hypothesis.

Zhang and coworkers were the first to suggest that HBO treatment during ischemia may influence the central cellular oxygen sensor system that controls hypoxia-inducible factor (HIF)-1α [161]. HIF is a posttranscriptionally regulated transcription factor that is continuously hydroxylated by prolyl hydroxylases, ubiquitinated and subsequently degraded by the proteasome under normoxic conditions [162]. During hypoxia, HIF-1α is stabilized and forms a heterodimer with HIF-1β, which enters the nucleus where it functions as a transcription factor for more than 100 genes, including those encoding erythropoietin, glucose transporters, glycolytic enzymes and vascular endothelial growth factor (VEGF) [162, 163].

It has been speculated that modification of the HIF pathway may contribute to the antiapoptotic effect of oxygen therapy [161]. In global cerebral ischemia, HBO-treatment led to a reduction of HIF-1α levels, which was associated with improved neuronal survival [159]. The same group reported similar findings in a subarachnoid hemorrhage model [160]. More recently we found, that HBO but not NBO treatment during focal cerebral ischemia reduced the expression of HIF-1α in the ischemic penumbra [112]. Furthermore, we observed, that the transcriptional activity of HIF-1α - as measured by VEGF mRNA expression - was significantly attenuated by HBO and NBO, respectively [112].

As most studies suggest that activation of the HIF pathway is a protective mechanism [162, 163], the reduction of HIF-1α and its downstream products by oxygen therapy appears paradoxical in view of its cerebroprotective effects in cerebral ischemia. But besides promoting neuroprotection [162, 163], some of the HIF target genes may also promote inflammatory proc-

esses [164, 165]. The role of HIF-1α signaling in cerebral ischemia, therefore, cannot be easily defined as either protective or deleterious. Intraparenchymal or intraventricular VEGF, for instance, independently decreased infarct size and reduced the number of TUNEL-positive apoptotic neurons, whereas endothelium-derived VEGF enhanced infarct volumes and lead to the development of cerebral edema by increasing BBB permeability [166-168]. As only few aspects of oxygen therapy-induced reduction of HIF-1α have been investigated at present, further investigation is warranted to clarify the complex HBO / HIF-1α interactions.

Oxygen Therapy as Bridging Option

With regard to recanalization therapy, we could demonstrate that - as monitored by MRI - early intraischemic HBO (3 ATA) therapy can immediately preserve tissue at risk in transient ischemia [67]. Suggesting that also NBO extends the reperfusion window, Kim *et al*. found smaller infarct volumes in rats after up to 3 h (NBO) versus 1 h (normoxia) of *transient* MCAO compared to the final infarct volume resulting from *permanent* vessel occlusion [87]. Similarly, Henninger and coworkers reported that NBO treatment results in a persistent PWI / DWI mismatch on MRI that could be salvaged by delayed (3 h) reperfusion [65].

Although we caution against uncritical translation of this data into the clinical setting, such bridging until reperfusion may have implications for the treatment of acute stroke because it could increase the volume of salvageable tissue for reperfusion therapies [67].

Preconditioning Paradigms and Regenerative Effects

Preconditioning refers to the adaptive response following a minor stress that improves the consequences of a subsequent pathological process such as ischemia. As the present review focuses on the therapeutic effects of oxygen therapy when administerd *after* ischemia-onset, preconditioning paradigms will only be discussed briefly. In several studies, preconditioning with HBO reduced infarct volumes and ameliorated neurological outcome in both transient [72, 73, 169] and permanent focal cerebral ischemia models [170] which was dose- and strain- dependent [169, 170]. After repeated HBO exposures, an increase in protein levels of the free radical scavenger manganese superoxide dismutase (MnSOD) and the anti-apoptotic Bcl-2 was observed in the hippocampus of gerbils that correlated with improved neuronal survival after ischemia [171, 172]. Freiberger and colleagues found a suppression of mitochondrial aconitase [173]. Accordingly, better tolerance to the oxidative stress generated by ischemia and reperfusion may underly HBO-induced preconditioning effects.

Little is currently known regarding the effects of oxygen therapy on tissue regeneration after stroke. Accumulating data show that multiple mechanisms of endogenous repair and remodeling are activated after stroke, and that the effects of a specific therapy may be different or even opposite dependent on the timing of its application [174]. Evolutionary preserved responses to brain injury suggest that cell death promoting processes during the acute phase of stroke might in fact account for neurovascular remodeling during the recovery stage [174]. Clinical observations of positive responses to oxygen therapy in chronic disease states have been reported repeatedly but the underlying therapeutic mechanisms are enigmatic [175]. Interestingly, Zhou *et al*. found that HBO-mediated improvement of neurological injury after global cerebral ischemia was correlated with a significant decrease in levels of the myelin-associated neurite outgrowth inhibitory protein Nogo-A, its receptor Ng-R, and its intracellular Rho GTPase signal pathway [176]. As neutralization of Nogo-A by monoclonal antibodies was shown to improve cortical plasticity and functional recovery after stroke [177, 178], the HBO-mediated mitigation of the Nogo-A pathways may be crucial in the process of vascular and cellular repair, respectively [176]. Clearly, extensive preclinical examination of the usefulness of oxygen therapy during the delayed phase after stroke is a prerequisite for application in human subacute or chronic ischemic stroke.

CONCLUSIONS

Over the past decade, multiple studies have clarified the potential and limitations of oxygen therapy in preclinical stroke models. Considering that the reduction of the infarct size amounts to 30-40%, the cerebroprotection induced by HBO is moderate. In the experimental setting, the effective time window of HBO initiation is only a few hours. Higher pressures (2.5-3 ATA) are more effective. Even though oxygen therapy has some effectiveness in permanent cerebral ischemia without vascular recanalization, it appears more promising for bridging of a transient ischemic period until reperfusion of the penumbra takes place. Therefore and due to its beneficial effects on secondary complications in the microcirculation a combination of HBO and reperfusion therapy seems reasonable. Compared to HBO, the implementation of NBO to the clinical setting would be substantially less demanding. Although recent experimental NBO-studies are promising, significant effectiveness of NBO was only shown in transient cerebral ischemia and if started within a narrow time window of maximum 30 minutes. Some studies suggest that the effect of HBO is superior to NBO both during transient and permanent cerebral ischemia, even if treatment initiation is delayed. However, this may be offset by the logistic advantages of NBO in human stroke. A sequential combination of both therapies is theoretically appealing but limited experimental studies do not support an additive of NBO and HBO at present.

While the therapeutic potential of oxygen therapy in ischemic stroke was considerably better defined over the past years, further studies are necessary to clarify the underlying cerebroprotective mechanisms. Recent studies have demonstrated that physical oxygen ther-

apy indeed improves oxygen supply of the ischemic penumbra as well as the cellular bioenergetic metabolism. Therefore, the mitochondria including their role in apoptotic cell death pathways as well as the modification of the cellular hypoxia sensor HIF-1α are considered as potential "downstream pathways" of oxygen therapy. Finally, its beneficial effects on the ischemic microcirculation suggest an important modification of various cell types within the neurovascular unit, which requires further characterization.

ABBREVIATIONS

HBO	=	Hyperbaric oxygen
NBO	=	Normobaric oxygen
ATA	=	Atmosphere absolute
MCAO	=	Middle cerebral artery occlusion
CBF	=	Cerebral blood flow
OEF	=	Oxygen extraction fraction
$CMRO_2$	=	Cerebral metabolic rate for oxygen
MRI	=	Magnetic resonance imaging
DWI	=	Diffusion-weighted MRI
PWI	=	Perfusion-weighted MRI
MRS	=	Magnetic resonance spectroscopy
MMP	=	Matrix metalloproteinase
COX	=	Cyclooxygenase
BBB	=	Blood-brain barrier
DNA	=	Deoxyribonucleic acid
HIF	=	Hypoxia-inducible factor
VEGF	=	Vascular endothelial growth factor
ROS	=	Reactive oxygen species

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