Normobaric oxygen treatment in acute ischemic stroke: a clinical perspective

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Abstract

Acute ischemic stroke is a common and serious neurological disease. Oxygen therapy has been shown to increase oxygen supply to ischemic tissues and improve outcomes after cerebral ischemia/reperfusion. Normobaric hyperoxia (NBO), an easily applicable and non-invasive method, shows protective effects on acute ischemic stroke animals and patients in pilot studies. However, many critical scientific questions are still unclear, such as the therapeutic time window of NBO, the long-term effects and the benefits of NBO in large clinic trials. In this article, we review the current literatures on NBO treatment of acute ischemic stroke in preclinical and clinical studies and try to analyze and identify the key gaps or unknowns in our understanding about NBO. Based on these analyses, we provide suggestions for future studies.

Key words: normobaric hyperoxia; oxygen therapy; stroke; ischemia; neuroprotection; oxidative stress; clinical studies; preclinical studies

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Introduction

The burdens of acute ischemic stroke (AIS) to patients, families and society are tremendous and cannot be over-emphasized. However, no effective neuroprotective agent has yet been developed to apply on AIS therapies.

Insufficient oxygen supply is critical in the pathophysiology of AIS. Oxygen therapy for AIS has been investigated for many years. Normobaric hyperoxia (NBO) is defined as breathing oxygen greater than 21%, up to 100%, at 1 absolute atmosphere pressure (ATA) (1 ATA = 101.325 kPa). NBO has been considered to have several advantages when compared to hyperbaric hyperoxia (HBO): NBO therapy is inexpensive and easy to administrate in ambulances or even at home prior to enroute a hospital after stroke. In recent years, interests increased in hyperoxia therapy, which elevates oxygen level in blood, have grown significantly (Stover, 2008). Because oxygen saturation of hemoglobin in healthy individuals at sea level already reaches 95% and above, NBO cannot further increase the amount of oxygen bound to hemoglobin. Therefore, the proportion of physically dissolved oxygen becomes more relevant. Given that the Bunsen solubility coefficient of oxygen in blood (37ºC) is 0.003 mL O_2/mmHg/mL blood. It was reported that NBO could increase the arterial partial pressure of oxygen (pO_2) from a mean of 84.1 to 345 mmHg (1 mmHg = 0.133 kPa) in the aorta, and from 60.9 to 154 mmHg in the smallest arterioles (Ivanov et al., 1999). Inhaling 100% oxygen at 45 L/min can raise the physically dissolved oxygen fraction from 0.3 mL to about 2 mL per 100 mL blood (Poli and Veltkamp, 2009). Furthermore, breathing 100% oxygen

could not only elevate pO_2 in blood but also in brain tissue (Sunami et al., 2000; Mulkey et al., 2001; Reinert et al., 2003; Niklas et al., 2004). Liu et al. (2006) reported that 95% NBO treatment during ischemia was able to maintain penumbral interstitial pO_2 close to the preischemic value in a rat model of ischemic stroke, while it may cause a two-fold increase in penumbral pO_2 level if given during reperfusion.

In the past several years, studies from our laboratory and others have demonstrated that NBO treatment effectively reduces both infarct volume and neurological deficits in rodents following AIS (Henninger et al., 2007; Tang et al., 2010; Chen et al., 2013). NBO has been also demonstrated to protect blood-brain barrier and extend the reperfusion time window in AIS animal models (Kim et al., 2005; Liu et al., 2008, 2009).

NBO *VERSUS* HBO IN EXPERIMENTAL ANIMAL MODELS AND HUMAN

In experimental animal models

Multiple studies have clarified potential and limitations of NBO treatment compared with HBO in preclinical stroke models. Many studies suggest that HBO treatment have neuroprotective effects regardless of whether HBO was initiated before onset of (Atochin et al., 2000; Miljkovic-Lolic et al., 2003) or during ischemia (Veltkamp et al., 2000, 2005; Yang et al., 2002; Hou et al., 2007) or even within a few hours of reperfusion (Yin et al., 2002, 2003) in transient cerebral ischemia. HBO was found to be harmful if started later than 12 hours of reperfusion (Badr et al., 2001; Lou et al., 2004). Furthermore, some studies have reported that the effect of HBO is superior to NBO during both transient and permanent cerebral ischemia (Weinstein et al., 1986, 1987). On the other hand, some articles have provided detailed evidence that HBO can induce oxidative stress in animal studies (Singhal, 2007; Liu et al., 2011). While controversial, they pointed out that oxidative stress induced by HBO therapy is due to highly concentrated, high pressure, or excessive duration of exposure to oxygen. Although, a few studies reported NBO therapy may also cause oxidative stress (Bigdeli et al., 2009), the likelihood of this seem to be low. Importantly, a recent paper that critically reviewed the current literature reports on the effect of NBO treatment on reactive oxygen species (ROS) and oxidative stress in AIS indicated that NBO does not increase ROS or oxidative stress if applied for a short duration (Weaver and Liu, 2015).

In human study

Although many clinical trials treated with HBO or NBO have been performed over the last 40 years, the usefulness of oxygen therapy in ischemic stroke remains an unresolved issue (Nighoghossian and Trouillas 1997; Bennett et al., 2005). In clinical trials for ischemia, HBO has shown mixed outcomes (Rusyniak et al., 2003; Bennett et al., 2014). The results of many early studies were mainly positive. But there were some shortcomings in those studies, including heterogeneous inclusion criteria, lacking comparison with control patients and blinded outcome assessment. There were three small randomized controlled clinical trials performed that showed little efficacy in ischemic stroke (Anderson et al., 1991; Nighoghossian et al., 1995; Rusyniak et al., 2003), probably because of inadequate sample size, a very long-time window, and delayed HBO therapy. Compared with HBO, NBO administration is easy and inexpensive. Therefore, it is surprising that so far only several human studies evaluating NBO for stroke treatment have been performed. More controlled studies are needed to determine the effectiveness of NBO treatment. To date, it is encouraging that no trials have shown any evidence that NBO is detrimental.

EVALUATING EFFECTS OF NBO IN ANIMAL EXPERIMENTS

NBO pretreatment before ischemia

NBO preconditioning (NBO treatment before ischemia) is worthy of recognition. Prior to filament-induced transient middle cerebral artery occlusion (MCAO), rats received NBO treatment continuously or intermittently (4 hours per day) for 4 days. After 60-minute ischemia with 24-hour reperfusion, NBO-treated rats showed less neurological deficits and infarct volume than the normoxic rats, with the upregulation of glutamate transporters in brain tissues and serum tumor necrosis factor-alpha level in blood. Interestingly, intermittent hyperoxia had relatively more significant effects on brain edema and blood-brain barrier protection than conventional NBO treatment (Bigdeli et al., 2007, 2008; Bigdeli and Khoshbaten, 2008). It is noteworthy that NBO pretreatment is effective but shows transient neuroprotective effects, which may disappear gradually 15 days after pre-treatment (Bigdeli et al., 2012).

These studies have suggested that NBO pretreatment may be applied to improve the outcomes for AIS patients, or for those who have had stroke precursors, such as transient ischemic attack (TIA) (Hadjiev and Mineva, 2010). Moreover, NBO pretreatment seems to be eligible for a direct translation into the human situation. However, before clinical use, further clinical trials are warranted to validate the short-term, long-term and potential side effects of NBO pretreatment.

NBO therapy post ischemia

Therapeutic time window of NBO

NBO treatment post ischemia is the most worthy of studying. Like the therapeutic time window of tissue-type plasminogen activator (tPA), NBO may also have a therapeutic window. Beynon et al. (2007) reported that NBO treatment

for 120 minutes, which started at 30 minutes post MCAO onset, failed to significantly decrease infarct volume in rats with 150-minute ischemia plus 7-day reperfusion. Another experiment also showed that 1-hour NBO treatment, which started at 60 minutes post reperfusion, could increase ischemia-induced brain damage in rats with 60-minute ischemia and 49-hour reperfusion (Haelewyn et al., 2011). These studies revealed that NBO treatment may not show neuroprotection but may be harmful if started later than 1 hour post ischemia for rats.

Moreover, the effects of NBO may be opposite in rats with different ischemic durations. It seems that NBO may not induce better outcomes in animals with long duration of ischemia. It was reported that NBO was only effective in transient ischemia rats and had a narrow time window of within 30 minutes post ischemia onset (Singhal et al., 2002). Therefore, in the future, it needs to verify the latest time points of NBO treatment that is still benefit to transient or permanent AIS animals.

Duration of NBO treatment

If started at early phase after stroke, both long and short duration of NBO have shown high neuroprotection in animal studies (Singhal, 2007; Michalski et al., 2011). A recent study compared the effects of different duration (2, 4, 6 and 8 hours) of NBO treatment, which started at 30 minutes post ischemia onset, on 90-minute ischemia and 22.5-hour reperfusion (Yuan et al., 2014). This study demonstrated that 8-hour NBO treatment exhibited the best neuroprotective capacity. Moreover, 8-hour NBO treatment not only attenuated oxidative stress during the period of reperfusion, but also alleviated apoptosis in ischemic penumbra in acute focal cerebral ischemic rats. However, it remains unclear whether the longer NBO lasts, the better the outcome would be. A study from Tatarkova et al. (2011) indicated that 60-hour NBO caused lipid and protein oxidative damage and stimulated superoxide dismutase expression and activity in mitochondria in the brain of guinea pigs. Therefore, the effects of long duration of NBO need further investigation to establish the optimal conditions for NBO treatment.

Future directions of NBO research with animal experiments

In transient ischemic animal models

It is encouraging that NBO shows neuroprotection in many transient experiments (Geng et al., 2013; Jin et al., 2013). However, we still need to figure out whether NBO may increase cerebral injury especially during reperfusion. Although some experiments indicated that NBO did not aggravate ischemic/reperfusion injury (Idani et al., 2012; Sun et al., 2014), very few literature reports specifically focused on the effects of NBO during the process of reperfusion. Interestingly, two reports demonstrated that NBO did not increase ROS production or oxidative stress when given during reperfusion (Liu et al., 2006; Rink et al., 2010). Importantly, a recent paper that critically reviewed the current literature reports on the effect of NBO treatment on ROS and oxidative stress with respect to AIS, concluded that NBO does not increase ROS or oxidative stress if applied for a short duration (Weaver and Liu, 2015). These studies would provide a basis for the decision of whether NBO therapy should be granted during reperfusion phase in transient ischemic stroke. Additionally, it is worth noting about an interesting study on the post-conditioning effects of NBO. It was reported that the neuroprotection exerted by intermittent NBO was greater than continuous NBO after 90 minute-ischemia and 72 hour-reperfusion, suggesting the mechanism of NBO is far beyond our understanding (Liu et al., 2012).

In permanent ischemic animal models

A very small proportion of stroke patients have the opportunity to receive the recanalization therapy in clinic due to delayed arrival. Therefore, permanent ischemia models are of importance to mimic the development of majority of stroke patients. However, most experiments showing positive effects of NBO are performed in transient stroke animals instead of permanent ischemic models. Therefore, there is a lot of work to do in permanent ischemic models. First, it is important to determine the latest time point of NBO treatment, on which NBO still show protective effects after stroke. This will provide the experimental evidence to support NBO's translational application in clinic. Second, we need to know how long NBO's protective effects could last, which will help optimize the strategies of NBO treatment on patients. Furthermore, it is important to find out whether the NBO treatment is still effective at the recovery phase after sub-acute or chronic ischemia.

Evaluating effects of NBO in clinical trials

To date, there are several literatures regarding NBO clinical trials in AIS patients. However, the conclusions are controversial, likely due to the difference in trial design (Michalski et al., 2011). As early as in 1999, a study with 550 AIS patients showed that NBO treatment did not improve 1-year survival, impairment scores and disability scores 7 months after stroke in the oxygen group (Ronning and Guldvog, 1999). There are several possible reasons for the lack of beneficial effects. The contributing factors might include the delayed NBO administration and low flow rate (at a rate of 3 L/min through a nasal catheter).

A NBO pilot study in the year of 2005 by Singhal et al. (2005) showed that patients' neuronal functions were

Table 1: Experimental studies of normobaric oxygen (NBO) treatment in acute ischemic stroke patients

Study	Inclusion criteria	Sample size	Treatment and duration	Outcome
Ronning and Guldvog (1999)	Within 24 hours after onset of a stroke	550	100% O_2, 3 L/min, 24 hours, by a nasal catheter	1-year survival, Impairment scores and disability scores 7 months after stroke: no differences
Singhal et al. (2005)	Nonlacunar, anterior circulation ischemic stroke < 12 hours after witnessed symptom onset or 15 hours after last seen neurologically intact; ineligible for intravenous/intra-arterial thrombolysis; modified Rankin Scale score < 1, a visual estimate for > 20% difference between diffusion weighted imaging (DWI) and mean transit time (MTT) lesion size	16	100% O_2, 45 L/min, 8 hours, by a simple facemask	National Institutes of Health Stroke Scale (NIHSS) scores (4 hours ↑, 24 hours↑, 1 week↑, 3 months: no differences) Relative stroke Infarct lesion volumes (4 hours↓)
Chiu et al. (2006)	(1) First-ever ischemic stroke (2) Infarction in the middle cerebral artery (MCA) presenting less than 48 hours after onset (3) NIHSS score of 12 (4) Modified Rankin Scale score no less than 4 (5) Brain CT images suggesting that the infarction area was more than one third of the MCA territory, and low-attenuation area of more than one third of the MCA territory on brain images within 48 hours after stroke	46	Inspired oxygen of 40%, 132.9 (range 48.0–168.5) hours, by a Venturi mask	Mortality and comorbidities ↓; overall length of stay was 17 days (median, 13 days; range, 3–65 days)
Singhal et al. (2007)	As mentioned above (Singhal et al., 2005)	6	100% O_2, 45 L/min, 8 hours, by a simple facemask	Magnetic resonance spectroscopic imaging: Lactate↓(4 hours during NBO); Lactate↑(post-NBO)
Gonzalez et al. (2010)	As mentioned above (Singhal et al., 2005)	14	100% O_2, 45 L/min, 8 hours, by a simple facemask	A stable mismatch for 4 or more hours for Patients who present outside the time window for thrombolytic therapy
Padma et al. (2010)	Anterior circulation ischemic strokes presenting within 12 hours of onset, ineligible for intravenous thrombolysis, NIHSS score of > 4, a MTT lesion larger than DWI, and an evidence of cortical hypoperfusion on magnetic resonance imaging	40	100% O_2, 10 L/min, 12 hours, by a simple facemask	Mean NIHSS scores (day 1, day 7, and month 3): no differences
Wu et al. (2012)	As mentioned above (Singhal et al., 2005)	16	100% O_2, 45 L/min, 8 hours, by a simple facemask	Ischemic lesion growth↓ (4 hours during NBO treatment)

improved as early as 15 to 20 minutes after NBO treatment, which were performed at the flow rate of 45 L/min *via* a facemask for 8 hours. Moreover, NBO transiently improved stroke scale scores and relative diffusion MRI lesion volumes of ischemia within 24 hours. However, there was no significant difference between NBO group and the control group (room air) at three months. A limitation of this study is a small sample size that only nine patients were in NBO group and seven patients in control group. Moreover, the reperfusion time of patients involved in the study were varied from 4 hours to 24 hours. According to the results from animal studies, NBO needs to be given as early as possible. Therefore, greater beneficial effects and more positive outcomes might be obtained if NBO therapy was applied within 4 hours after the onset of symptoms.

Another human study in 2006 documented that there were less mortality and comorbidities in severe AIS patients who breathed 40% oxygen by a Venturi mask for 132 hours (Chiu et al., 2006). In this study, the sample size (46 patients in total) was a little larger than the Singhal et al.'s study. However, the criteria of inclusion (patients within 48 hours after stroke) were rather broad, and breathing gas was the mixed oxygen with air (40% O_2).

In 2007, another study of Singhal et al. (2007) with six patients indicated that NBO was able to decrease lactate using magnetic resonance spectroscopy imaging by a voxel-based analysis. The mean N-acetyl-aspartate levels from baseline to 4 hours was different between NBO and control groups, showing that NBO improves aerobic metabolism and preserves neuronal integrity in the ischemic brain.

In 2010, Singhal group further reported that eight patients who presented outside the time window for thrombolytic therapy had a larger diffusion/perfusion mismatch on MRI for 4 hours or more after NBO treatment, compared with

six patients with normoxia (Gonzalez et al., 2010). All were enrolled in a NBO pilot study in the year of 2005 above mentioned. These data came from 14 patients with 8 patients receiving NBO treatment and 6 patients with normoxia. National Institutes of Health Stroke Scale (NIHSS) showed no significant correlation between the two groups.

In 2012, Wu et al. (2012) reported a clinical study with 16 patients who underwent less than 12 hours after witnessed stroke onset or 15 hours after last seen neurologically intact. They observed that NBO group had a > 20% visually-estimated mismatch in acute diffusion weighted imaging and perfusion-weighted imaging lesion volumes. This study showed that NBO had therapeutic potential because ischemic lesion growth was attenuated with 4-hour NBO treatment.

On the other hand, some clinical trials failed to show beneficial effects of NBO. A recent study in Indian patients with AIS indicated that NBO did not improve the clinical scores of stroke outcome (Padma et al., 2010). The contributing factors for the lack of effect might include the delayed NBO administration (on an average 6–10 hours after stroke onset) and lower flow rates (12 L/min, *versus* 45 L/min used in the study of Singhal et al. (2005)).

Therefore, the results of clinical trials were much more complicated than the animal studies, due to the difference in sample size, the starting time point of NBO treatment and differential patient inclusion criteria. The outcomes of NBO treatment on patients are summarized in **Table 1**.

The future direction of clinical trials

As we mentioned earlier, NBO is easy to apply. However, NBO treatment in all published clinical trials was performed in hospital after the patients had reached there. The average starting time of NBO administration was 6−10 hours, or more, after onset of ischemia. We look forward to the clinical trials designed for NBO treatment as early as in the ambulance. Moreover, determining the therapeutic time window of NBO treatment is critical in clinic. We need to determine how long after ischemia NBO treatment is still of beneficial effects. In addition, NBO preconditioning could be applied to TIA patients, who are at high risk of stroke and 1/3 of whom is likely to develop into AIS. Finally, research on the efficacy of NBO in sub-acute and chronic patients is warranted.

Conclusions

As a non-pharmaceutical and non-invasive treatment, NBO is worthy of notice as a promising AIS therapy. NBO shows protective effects not only in basic animal experiments but also in pilot clinical trials. Further preclinical and clinical studies are needed to determine its optimum therapy time window and long-term effects. Large clinical studies with optimal inclusion criteria of patients and the strategies of NBO treatment are warranted in the future.

Author contributions

SHS reviewed the NBO studies in stroke, wrote up the manuscript. ZFQ and YML improved the structure. XMJ reviewed the NBO studies in clinic and edited the overall manuscript. KJL participated in the overall design of the review and obtained partial funding. All authors have read and approved the final version of this paper.

Conflicts of interest

The authors declare that they have no competing interests.

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