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Effects of stroke severity and treatment duration in normobaric hyperoxia treatment of ischemic stroke

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Abstract

In order to improve clinical trial design and translation of normobaric oxygen (NBO) treatment of ischemic stroke, NBO treatment parameters need to be better understood. This study investigated NBO treatment efficacy at two different stroke severities and two NBO treatment durations in rats. For the 60-min middle cerebral artery occlusion (MCAO), NBO treatment for 25 mins and 150 mins were studied. For the 90-min MCAO, NBO treatment for 55 mins and 150 mins were studied. Cerebral blood flow (CBF), apparent diffusion coefficients (ADC) and T2 MRI were acquired during occlusion prior to treatment, after reperfusion, and 48 hours after MCAO. The effects of NBO treatment on lesion volumes, and CBF, ADC and T2 of ischemic core, perfusion-diffusion mismatch and normal tissue were analyzed longitudinally. The major findings were: i) NBO treatment was effective in both groups of stroke severities, salvaging similar percentage of initial abnormal ADC tissue, and ii) NBO treatments continued after reperfusion were more beneficial than NBO treatment during occlusion alone for both MCAO groups. These findings underscore the importance of the effects of NBO duration and stroke severity on treatment outcomes.

Keywords

NBO; ischemic penumbra; cerebral ischemia; reperfusion; MRI; diffusion; arterial spin labeling

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The authors declare no conflict of interest.

Compliance with Ethical Standards

All applicable institutional guidelines for the care and use of animals were followed.
Human studies: not applicable.

Introduction

Normobaric oxygen (NBO) treatment has the potential to improve brain tissue oxygenation and rescue hypoxic tissue following ischemic stroke (Shin et al., 2007). Many studies have shown that NBO treatment reduced histological infarct volume and improved behavioral function in experimental stroke (Henninger et al., 2007; Henninger et al., 2009; Jin et al., 2013; Kim et al., 2005; Liu et al., 2012; Singhal et al., 2002a; Singhal et al., 2002b; Singhal et al., 2007; Sun et al., 2010; Sun et al., 2011; Sun et al., 2014). The mechanisms of NBO-mediated neuroprotection in stroke include improved tissue oxygenation (Shin et al., 2007), improved aerobic metabolism (Singhal et al., 2007), and reduced blood-brain barrier damage (Liu et al., 2009), reduced free radical damage (Yuan et al., 2010) and reduced peri-infarct depolarization (Shin et al., 2007). However, a few studies reported NBO treatment worsened stroke outcomes, including increased infarct volume (Haelewyn et al., 2011) and white matter damage (Mickel et al., 1987; Mickel et al., 1990). In a phase I clinical trial, NBO yielded transient improvements in MRI and clinical deficits. In the absence of arterial recanalization, 8-hour NBO treatment significantly improved relative cerebral blood flow (CBF) within the ischemic regions at 4 and 24 hours. However, these improvements were reported to be transient (Singhal et al., 2005). Subsequent phase II clinical trial (NCT00414726) was terminated due to imbalance in deaths favoring control arm, although deaths were not attributed to treatment by the blinded external medical monitor. Future clinical trial in NBO treatment of stroke is likely. In order to better inform future NBO stroke trials, NBO treatment parameters need to be better understood. Some of these NBO treatment parameters include when to start treatment (early versus late treatment), during which ischemic phase to treat (during occlusion versus after reperfusion), how long to treat (several hours to days), and which patient groups will likely benefit (i.e., patients with mild versus severe stroke, and patients with versus without substantial perfusion-diffusion mismatch (Albers, 1999; Fisher, 2003; Kidwell et al., 2003; Warach, 2003)).

Amongst the NBO treatment studies in experimental ischemic stroke, only a few used MRI to monitor tissue fate longitudinally before and during treatment. Singhal et al. found that NBO treatment of permanent middle cerebral artery occlusion (MCAO) in rats minimized apparent diffusion coefficients (ADC) abnormalities during the acute phase and reduced histological infarct volume at 48 hours, as compared to the air group (Singhal et al., 2002a). Henninger et al. found that NBO treatment in both permanent and transient MCAO models reduced ADC abnormalities and stopped the progression of perfusion-diffusion mismatch during occlusion. At 24 hours, both NBO groups showed significantly smaller histologically defined lesion volumes compared to the air group (Henninger et al., 2007). Both of these studies investigated the NBO treatment only during occlusion and the effects of NBO treatment extending *after* reperfusion on tissue ADC, CBF and T2 are unknown.

In this study, we aimed to address two specific NBO treatment parameters mentioned above, namely: is NBO treatment more effective in mild or severe ischemic stroke, and is NBO treatment more effective given during occlusion only or extended beyond reperfusion? Serial multimodal MRI was used to study two different degrees of stroke severity (60-min and 90-min occlusion) in a rat MCAO model, and two NBO treatment durations (during occlusion only and extended after reperfusion) for each of the MCAO groups. In the 60-min

MCAO group, 25-min and 150-min NBO treatments were studied. In the 90-min MCAO model, 55-min and 150-min NBO treatments were studied. NBO treatments started 35 mins after MCAO following the initial pre-treatment MRI. CBF, ADC and T2 measurements were made prior to treatment, during occlusion, after reperfusion and 48 hours after MCAO. The primary readout was lesion volume at 48 hours and secondary readouts are quantitative CBF, ADC and T2 of the core, mismatch and normal tissue at different time points. *Our central hypotheses are that NBO treatment is effective in reducing infarct volume for both stroke severities, and NBO treatment extending after reperfusion is more beneficial in reducing infarct volume than NBO treatment during occlusion alone.*

Results

The randomized, vehicle controlled and double-blinded experimental design is shown in Figure 1. Respiration rate (58 ± 3 bpm), heart rate (350–450 bpm), and arterial oxygen saturation (95 ± 4 %) by oximetry were within normal physiological ranges unless otherwise perturbed by NBO, consistent with stroke studies under similar experimental preparations from our laboratory (Shen et al., 2003; Shen et al., 2004b; Shen et al., 2005; Shen et al., 2014; Sicard et al., 2006a; Sicard et al., 2006b; Tanaka et al., 2007).

In the contralesional homologous regions, CBF and ADC values were within normal physiological ranges and they were not statistically different amongst groups (data not shown), consistent with previous findings from our laboratory (Shen et al., 2003; Shen et al., 2004b; Shen et al., 2005; Shen et al., 2014; Sicard et al., 2006a; Sicard et al., 2006b; Tanaka et al., 2007). Percent changes in ADC and CBF were thus analyzed with respect to the homologous regions in the contralesional hemisphere for comparison across time points.

NBO treatment following 60-min MCAO

The effects of NBO on lesion volume on MRI images are shown in Figure 2A & 2B

At 30 mins after MCAO, ADC and CBF lesion volumes prior to treatments were similar amongst the three groups. Perfusion deficit volumes were larger compared to ADC deficit volumes, confirming the presence of a perfusion-diffusion mismatch in all three groups. At 48 hours after occlusion, T2-derived lesion volume was smallest in the 150-min NBO group followed by the 25-min NBO and the air group.

The group lesion volume data are shown in Figure 2C. At 30 mins, the CBF lesion volumes of the three groups were not significantly different from each other (343 ± 44 , 339 ± 20 285 ± 40 mm³, respectively, $P > 0.05$) and all were significantly larger than the ADC lesion volumes ($P < 0.05$), confirming the presence of a perfusion-diffusion mismatch in all three groups. All three groups started with similar abnormal ADC lesion volumes before NBO treatment. After reperfusion (90 mins post occlusion), lesion volumes dropped significantly in all groups and remained reduced at 180 mins. At 48 hours, 150-min NBO group showed significantly smaller lesion volume compared to the air and 25-min NBO group.

Figure 2D summarizes the percent changes in lesion volume between 48 hours and 30 mins after MCAO for the three groups. The lesion volume of the air and 25-min NBO group

increased by $19 \pm 6\%$ and $4 \pm 2\%$, respectively, whereas the lesion volume of the 150-min NBO group decreased by $-18 \pm 5\%$. The changes in lesion volumes were statistically different between 150-min NBO and air group ($P < 0.001$), between 150-min NBO and 25-min NBO group ($P < 0.01$). However, no statistical significance was found between the 25-min NBO and air group.

The effects of NBO on CBF were analyzed (Figure 2E)

At 30 mins (during MCAO), the core CBF values were below the ischemic threshold (0.35 ml/g/min) (Meng et al., 2004; Shen et al., 2003)) in all three groups and CBF values were not significantly different from each other ($P > 0.05$). At 90 mins (after reperfusion), core CBF values improved significantly in all three groups, with better improvement seen in the 150-min NBO and 25-min NBO groups. At 180 min, CBF values were slightly reduced relative to those at 90 min, and CBF were still differentially higher in the 150-min NBO and 25-min NBO groups compared to air. Similar patterns were observed in the mismatch CBF data. All CBF values in core clusters were below normal after reperfusion.

The effects of NBO on ADC are shown in Figure 2F

At 30 mins (during MCAO), core ADC values were markedly reduced in all three groups and ADC values were not significantly different from each other ($P > 0.05$). At 90 mins (after reperfusion), core ADC values improved significantly in all three groups but were below normal values. At 180 mins, the patterns did not change significantly. In the mismatch pixels, ADC values were only mildly reduced from normal and reperfusion improved ADC slightly. There were no significant differences in mismatch ADC amongst groups ($P > 0.05$).

NBO treatment following 90-min MCAO

The effects of NBO on lesion volume are shown in Figure 3A & 3B. At 30 mins after MCAO, ADC and CBF lesion volumes prior to treatments were similar amongst the three groups. Perfusion deficit volumes were larger compared to ADC deficit volumes in all groups, indicating the presence of a perfusion-diffusion mismatch. At 48 hours after MCAO, the T2-derived lesion volumes were smallest in the 150-min NBO group followed by the 55-min NBO and air group.

The group lesion volume data are shown in Figure 3C. At 30 mins, the CBF lesion volumes of the three groups were not significantly different from each other (277 ± 35 , 267 ± 26 , 295 ± 36 mm³, respectively, $P > 0.05$) and all were significantly larger than the ADC lesion volumes ($P < 0.05$), confirming the presence of a perfusion-diffusion mismatch in all three groups. At 30 mins, all groups started with similar ischemic lesion volumes. At 60 min, lesion volumes significantly dropped in 55-min NBO group and 150-min NBO group, whereas the lesion volume trended higher in air group. At 120 and 180 mins (after reperfusion), the lesion volumes dropped in all groups, with a larger drop in the 150-min NBO followed by 55-min NBO group compared to the air group. At 48-hour, the 55-min and 150-min NBO groups showed significant reduction in lesion volume compared to the air group.

Figure 3D summarizes the percent changes in lesion volume between 30 mins and 48 hours after MCAO. The lesion volume in the air group increased by $17\pm7\%$ ($P<0.05$), whereas the lesion volumes in the 55-min NBO and 150-min NBO group decreased by $-11\pm5\%$ ($P<0.05$) and $-34\pm4\%$ ($P<0.05$), respectively. Difference in lesion volume growth was statistically significant between 150-min NBO and air group ($P<0.001$) and between 55-min NBO group and air group ($P<0.01$).

The effects of NBO on CBF are shown in Figure 3E. At 30 and 80 mins (during MCAO), the core CBF values were below the ischemic threshold in all three groups. At 180 mins (after reperfusion), core CBF values improved significantly in all three groups, and there were no significant differences amongst groups ($P>0.05$). Similar patterns were observed in the mismatch CBF data.

The effects of NBO on ADC are shown in Figure 3F. The core ADC values were markedly reduced at 30 mins after MCAO and further reduced at 80 mins after MCAO. At 180 mins (after reperfusion), core ADC values only improved slightly, with better improvement seen in the 150-min NBO group followed by 55-min NBO group compared to the air group. In the mismatch, ADC values were only mildly reduced and reperfusion only improved the mismatch ADC slightly. There were no significant differences in mismatch ADC amongst groups ($P>0.05$).

Lesion volume between MCAO groups

Although trends existed, there were no significant differences in lesion volumes at 180 mins and 48 hrs after stroke onset between the 60-min MCAO and 90-min MCAO.

Discussion

This study used multimodal MRI to investigate the effects of stroke severity and treatment duration in normobaric hyperoxia treatment of ischemic stroke by longitudinally tracking the evolution of ischemic brain injury in different ischemic tissue types. The major findings are: i) the longer (150 mins versus 25 mins NBO, and 150 mins versus 55 mins NBO) NBO treatments were more effective in reducing 48-hr infarct volume compared to air, whereas the shorter (25 mins) NBO treatment only showed trend of reducing infarct volume, ii) NBO treatment extending after reperfusion (up to 150 mins) was beneficial in reducing infarct volume at 48 hours, iii) Compared to the air group, NBO treatment stopped the progression of ADC lesion volume (for shorter 25min NBO treatment) or reversed the initial ADC lesion volume (for longer 55 mins and 150 mins NBO treatment), and iv) NBO treatment did not show consistent significant improvement in ADC or CBF in the mismatch and core pixels although some trends of improvements were present. These results support our central hypotheses that NBO treatment is effective in reducing infarct volume at both stroke severities, and NBO treatment extending after reperfusion is more beneficial than NBO treatment during occlusion alone.

Effects of NBO on lesion volume

Compared to the air group, NBO treatment stopped the progression of ADC lesion volume (for shorter treatment) or reversed the initial ADC lesion volume (for longer treatment).

Such improvement in ADC lesion volume persisted after reperfusion. At 48 hours, all four NBO treatment groups yielded smaller infarct volumes compared to the corresponding air groups. Moreover, NBO treatment reversed ADC abnormality in three out of the four NBO treatment groups. The exception was the 25-min NBO group, which only showed reduced infarct volume relative to air group, but did not show reversed ADC abnormality. A likely explanation is that the NBO treatment was too short to reverse ADC abnormality although it was still beneficial. Our results are in general agreement with two previous MRI studies, which found NBO treatment reduced infarct volumes (Henninger et al., 2007; Singhal et al., 2002a). Note that NBO can only delay ischemic brain injury and buy time and tissue reperfusion is ultimately needed to improve outcomes. NBO treatment *per se* is unlikely to be effective if subsequent reperfusion is absent or delayed (Beynon et al., 2007; Li et al., 1994).

Some older studies found NBO, especially during reperfusion, exacerbates reperfusion injury, increases oxidative stress and worsens outcomes (Haelewyn et al., 2011; Mickel et al., 1987; Mickel et al., 1990), including white matter damage (Mickel et al., 1987; Mickel et al., 1990). A few studies have reported no benefit of NBO treatment, which may be attributed to delayed onset of the NBO treatment (Fujiwara et al., 2009; Haelewyn et al., 2011; Singhal et al., 2002a). More recent studies generally reported NBO treatment does not increase oxidative stress in cerebral ischemia and does improve outcomes (Kim et al., 2005; Liu et al., 2006; Singhal et al., 2002b). Our findings are consistent with those found NBO to be beneficial. Our data not only showed NBO treatment extending after reperfusion did not increase infarct volume, but also that NBO treatment extending after reperfusion was more effective in reducing infarct volume compared to NBO treatment only during occlusion.

An advantage of serial MRI approach is that the initial ADC lesion volumes at 30 mins after MCAO pre-treatment were verified to be identical between the air and NBO groups in the same animals. Moreover, the presence of the perfusion-diffusion mismatch was also confirmed. This concept is relevant to proper patient selection in the clinics to improve specific treatment efficacy (Albers, 1999; Fisher, 2003; Kidwell et al., 2003; Warach, 2003).

Effects of NBO on CBF

In the 90-min MCAO group, NBO treatments did not affect CBF in the mismatch and core pixels. Previous MRI studies that investigated longer occlusion durations also reported similar findings (Henninger et al., 2007; Singhal et al., 2002a). Another study found that NBO treatment significantly improved penumbral CBF but not core CBF in 90-min MCAO, where the “penumbral tissue” was defined using Laser Doppler Flowmetry (Liu et al., 2006).

In the 60-min MCAO group, NBO treatment improved CBF in the mismatch and core pixels after reperfusion. However, these improvements were seen only for animals receiving NBO during occlusion. In the 60-min MCAO duration, the NBO treatment was likely too short to improve CBF in a sustained way in the ischemic tissue. The effects of NBO treatment extending after reperfusion on CBF have not been widely or systematically studied previously using MRI to monitor longitudinally changes. A novel finding of this study is that NBO improved tissue perfusion but only in less severe stroke group. It is likely that

cerebral vessels was less damaged in shorter MCAO duration, thereby more amenable to improved reperfusion. These findings also showed that NBO administration after reperfusion did not negatively affect CBF.

Effect of NBO on ADC

Compared to the air group, NBO treatments showed slight trends of improved ADC in the mismatch and core pixels during occlusion and after reperfusion but not consistently across different treatment and MCAO groups. Previous studies have looked at longer occlusion periods and report significant improvements in the ADC of the mismatch tissue as a result of NBO treatment (Henninger et al., 2007; Singhal et al., 2002a). Singhal et al. reported that NBO (started at 45-min after MCAO) did not affect striatal ADC but significantly improved cortical ADC up to 120 mins after occlusion (Singhal et al., 2002a). Henninger et al. found that NBO (started at 30 mins after MCAO) did not affect core ADC but significantly improved mismatch ADC at 150 and 180 mins after occlusion (Henninger et al., 2007). Improvements in ADC likely result from improved tissue oxygenation (Shin et al., 2007) and aerobic metabolism (Singhal et al., 2007) following NBO administration in the acute phase, which translated into reduced infarct volume at 48 hours.

Effects of stroke severity

We chose the MCAO durations based on our previous MRI studies. In our models, most mismatch disappears by ~3 hours after MCAO in the absence of reperfusion (Shen et al., 2003; Shen et al., 2004a). We are cognizant that different laboratories reported slightly different lesion volumes and mismatch dynamics for identical MCAO durations, which could be due to differences in anesthetics, duration of anesthesia, and occluder, among others. The hyperacute phase in rats is ~3 hours of stroke onset in our stroke model, significantly shorter than that of humans (Heiss, 2000) due to species differences, among others.

Although the ADC lesion volume of the 60-min MCAO group was (albeit not significantly) smaller than that of 90-min group at 180 mins after stroke, the infarct volume at 48 hours were not significantly different between the two MCAO groups, consistent with our previous report (Shen et al., 2013b). A possible explanation is that large variation of infarct volumes that resulted in overlapping infarct sizes. Nonetheless, NBO is effective in reducing infarct volume in both MCAO groups, with dependence on the durations of treatment. NBO improved tissue perfusion but only in less severe ischemia. It is likely that cerebral vessels were less damaged in shorter MCAO duration, thereby more amenable to improved reperfusion.

Effect of NBO duration

A major finding of our study is that the longer (150 mins versus 25 mins NBO, and 150 mins versus 55 mins NBO) NBO treatments were more effective in reducing 48-hr infarct volume compared to air, whereas the shorter (25 mins) NBO treatment only showed trend of reducing infarct volume, supporting the notion that NBO treatment improved tissue oxygenation (Shin et al., 2007), improved aerobic metabolism (Singhal et al., 2007), reduced blood-brain barrier damage (Liu et al., 2009), reduced free radical damage (Yuan et al.,

2010) and reduced peri-infarct depolarization (Shin et al., 2007). NBO treatment might also overcome possible negative effects of reperfusion injury in the presence of NBO. Our results are in general agreement with previous MRI and histological studies of NBO treatments. Henninger et al. found that a 6-hour NBO treatment reduced lesion volume by 44%, whereas a 3-hour NBO treatment caused a 10% reduction in permanent MCAO rats (Henninger et al., 2007). Liu et al. reported that a 70-min NBO treatment significantly reduced 24-hour lesion volume whereas 18-min NBO treatment failed to show any improvement over air group in 90-min rat MCAO model (Liu et al., 2012). Both of these studies reported lower 24-hour histologically defined lesions for longer NBO treatment. Previous MRI studies investigated the ADC and CBF changes only during occlusion. Our study investigated the NBO treatment *after* reperfusion on tissue ADC, CBF and T2. These results support the notion that NBO treatment after reperfusion has beneficial effects in reducing infarct volume at 48 hours after stroke.

Limitations and future perspectives

A limitation of this study is that measurements were made only up to 48 hours as this study focused on evaluating NBO treatment on acute stroke. Future studies will investigate NBO treatment in chronic stroke, along with behavioral assessments and histological validation, to determine whether such improvement is transient or permanent. Another limitation is that the 60-min and 90-min MCAO induced similar stroke severity and future studies could explore additional occlusion durations as well as additional treatment durations. NBO is safe, cost-effective and has the potential for early ‘doorstep-to-clinic’ intervention to preserve ischemic tissues and extend time window for reperfusion strategies. NBO treatment may also be combined with other treatments to further improve neuroprotection in acute stroke.

Conclusions

This study used multimodal MRI to track the evolution of ischemic brain injury in different tissue types under two different NBO treatment durations in rats subjected to two MCAO durations. These results support our central hypotheses are that NBO treatment is effective in reducing infarct volume in both stroke severities, and NBO treatment extending after reperfusion is more beneficial than NBO treatment during occlusion alone.

Methods

Animal Preparation

All experimental procedures and writing followed the ARRIVE guidelines. All experimental procedures were approved by the Institutional Animal Care and Use Committees of the University of Texas Health Science Center San Antonio. Animals were housed in a 12-hour light/dark cycle with food and water ad libitum. Focal cerebral ischemia of the right hemisphere was induced by MCAO using intraluminal filament (0.35–0.37 intraluminal silicon rubber-coated filaments (Docol Corporation, Massachusetts USA) inserted via the external carotid artery in 42 healthy male Sprague-Dawley rats (250–300 g, 8–10 weeks, Charles River Laboratories) under 2% isoflurane (Shen et al., 2005). Reperfusion was

achieved by completely withdrawing the occluder which was performed with the animal taken out of the holder.

Animals were mechanically ventilated (Model 683, Harvard Apparatus, South Natick, MA) and secured in supine position using a MRI compatible rat stereotaxic headset, and maintained at 1.2% isoflurane during MRI scans. End-tidal CO₂, rectal temperature, heart rate and arterial oxygenation saturation were recorded and maintained within normal physiological range during MRI experiments. Imaging was performed during MCAO and animal holder was slid out on the rail to withdraw the filament for reperfusion (Shen et al., 2013a). Five animals with unsuccessful MCAO or unsuccessful reperfusion, which were determined by absence or small size (i.e., less than 50 mm³) of ADC lesion at 30 mins and absence of significant CBF recovery by MRI after withdrawal of the occluder respectively, were excluded from the study.

Animal Groups

Administration of NBO or air to animals was randomized prior to starting of the study. The timelines of MCAO, NBO administration, reperfusion and MRI experiments are shown in Figure 1. Two MCAO durations were studied. In the 60-min MCAO group, animals were treated with 25 mins of NBO (n=5, group I), 150 mins of NBO (n=8, group II), or air (n=6, group III). In the 90-min MCAO group, animals were treated with 55 mins of NBO (n=6, group IV), 150 mins of NBO (n=6, group V), or air (n=6, group VI). NBO administration was started ~35 mins after MCAO immediately after the pre-treatment ADC scan.

MRI Experiments

MRI experiments were performed on a 7 Tesla Bruker Biospec scanner with a 76 G/cm BGA12S gradient insert (Billerica, MA). Custom made brain and neck surface coils were used for imaging and perfusion labeling (Shen et al., 2005; Shen et al., 2013a). Apparent diffusion coefficient (ADC) was measured using spin-echo diffusion-weighting gradients with echo planar readout. The diffusion gradients were applied separately along the x, y and z direction. Two b values of 4 and 1200 s/mm² were used. CBF measurements were performed using two coil continuous arterial spin labeling (cASL) technique with gradient echoplanar readout. The cASL sequence used a 2.7 ms square labeling RF pulse. T2 maps were acquired using fast spin echo with four effective echo times (25, 40, 75 and 120 ms), echo train length (ETL) = 8, and 4 signal averages. Other MRI scan parameters included: seven slices, slice thickness=1.5mm, single shot EPI, matrix size = 96×96 (reconstructed to 128×128), field of view = 25.6×25.6mm², flip angle = 90°, repetition time (TR) = 3s, echo time (TE) = 10.2 ms for CBF and 30 ms for ADC (Shen et al., 2003).

In the 60-min MCAO animals, serial ADC and CBF maps were acquired at 30, 90, 180-min and 48-hours post MCAO. T2 maps were acquired at 48-hours post MCAO. In the 90-min MCAO animals, serial ADC and CBF maps were acquired at 30, 80, 180-min and 48-hours post MCAO. Additionally, ADC maps were also acquired at 120-min post MCAO. T2 maps were acquired at 48-hours post MCAO.

Data Analysis

ADC, CBF and T2 maps were calculated using Matlab codes (Shen et al., 2005). Images were co-registered using QuickVol II and ImageJ (Liu et al., 2004; Shen et al., 2005). Three tissue types (normal, perfusion-diffusion mismatch and ischemic core) were classified based on 30-min (pre-treatment) ADC deficit and CBF deficit using pre-defined thresholds. The mean T2 value from the normal hemisphere plus two times the standard deviation was used as the threshold for infarcted tissue for the 48-hour T2 maps. Threshold values of ADC ($0.53 \times 10^{-3} \text{ mm}^2/\text{s}$) and CBF (0.35 ml/g/min) were used to define the ischemic core and perfusion deficit respectively (Meng et al., 2004; Shen et al., 2003). Perfusion diffusion mismatch (ischemic penumbra) was defined as the difference between perfusion deficit and ADC defined core.

ADC and CBF values of core, mismatch and contralesional hemisphere (left hemisphere) were measured using Stimulate (University of Minnesota). Initial lesion volume was defined as the volume of the pre-treatment ADC deficit at 30 mins after occlusion. Final lesion volume was defined using T2 map at 48 hours after occlusion using the following formula: corrected lesion volume = uncorrected lesion volume – (right hemisphere – left hemisphere) (Meng et al., 2004; Shen et al., 2013a)

Statistical analysis

ADC and CBF values for different tissue groups (core, mismatch and contralesional tissue) were tracked longitudinally over time. One-way ANOVA with Tukey's post-hoc test was used for comparison across treatment groups. Two-tailed paired t-test was used for 'before-after' comparisons within the same group. A p value of 0.05 was taken to be statistically significant. All values are reported as mean $\pm$ SEM unless stated otherwise.

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- Normobaric oxygen (NBO) treatment parameters could affect stroke outcomes.
- Short NBO treatment duration stops the progression of lesion volume.
- Longer NBO treatment duration reversed the initial lesion volume.
- NBO treatment is effective in reducing infarct volume at two different stroke severities
- NBO treatment extending after reperfusion is more beneficial than only during occlusion alone

Figure 1.
Experimental designs of NBO treatment groups for 60- and 90-min MCAO animals.

Figure 2.

60-min MCAO group: (A) Representative ADC, CBF and T2 maps at 30 mins and 48 hours after occlusion for air, 25-min NBO and 150-min NBO groups. Only one image slide is shown for clarity. Images from each group were obtained from the same animals. (B) ROIs were overlaid to delineate the lesion volumes. (C) Progression of ADC-defined lesion volumes in air, 25-min NBO and 150-min NBO groups. The data points in the box were those of CBF-defined lesion volumes at 30 mins during occlusion. (D) Percent growth of initial infarction at 48 hours for air, 25-min NBO and 150-min NBO groups. (E) Mean CBF values represented as percent of normal (contralateral hemisphere) values in core and mismatch pixels at 30, 90 and 180 mins after occlusion for air, 25-min NBO and 150-min

NBO groups. **(F)** Mean ADC values represented as percent of normal (contralesional hemisphere) values in core and mismatch pixels at 30, 90 and 180 mins after occlusion for air, 25-min NBO and 150-min NBO groups. All error bars are standard errors of the means.

Figure 3.

90-min MCAO group: (A) Representative ADC, CBF and T2 maps at 30 mins and 48 hours after occlusion for air, 55-min NBO and 150-min NBO groups. Images from each group were obtained from the same animals. (B) ROIs were overlaid to delineate the lesion volumes. (C) Progression of ADC-defined lesion volumes in air, 55-min NBO and 150-min NBO groups. The data points in the box were those of CBF-defined lesion volumes at 30 mins during occlusion. (D) Percent growth of initial infarction at 48 hours for air, 55-min NBO and 150-min NBO groups. (E) Mean CBF values represented as percent of normal (contralateral hemisphere) values in core and mismatch pixels at 30, 80 and 180 mins after occlusion for air, 55-min NBO and 150-min NBO groups. (F) Mean ADC values represented as percent of normal (contralateral hemisphere) values in core and mismatch

pixels at 30, 80 and 180 mins after occlusion for air, 55-min NBO and 150-min NBO groups. All error bars are standard errors of the means.