The Efficacy of Hyperbaric Oxygen Therapy on Middle Cerebral Artery Occlusion in Animal Studies: A Meta-Analysis

Yang Xu¹, Renjie Ji¹, Ruili Wei¹, Bo Yin², Fangping He¹, Benyan Luo¹*

¹ Department of Neurology, Brain Medical Centre, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, ² Department of Neurology, Renmin Hospital of Wuhan University, Wuhan, China

* luobenyan@zju.edu.cn

Abstract

Background

Inconsistent results have been reported for hyperbaric oxygen therapy (HBO) for acute stroke. We conducted a systematic review and meta-analysis to evaluate the benefit of HBO in animal studies of middle cerebral artery occlusion (MCAO).

Methods

A systematic search of the literature published prior to September 2015 was performed using Embase, Medline (OvidSP), Web of Science and PubMed. Keywords included “hyperoxia” OR “hyperbaric oxygen” OR “HBO” AND “isch(a)emia” OR “focal cerebral ische-mia” OR “stroke” OR “infarct” OR “middle cerebral artery occlusion (MCAO).” The primary endpoints were the infarct size and/or neurological outcome score evaluated after HBO treatment in MCAO. Heterogeneity was analyzed using Cochrane Library’s RevMan 5.3.5.

Results

Fifty-one studies that met the inclusion criteria were identified among the 1198 studies examined. When compared with control group data, HBO therapy resulted in infarct size reduction or improved neurological function (32% decrease in infarct size; 95% confidence interval (CI), range 28%–37%; p < 0.00001). Mortality was 18.4% in the HBO group and 26.7% in the control group (RR 0.72, 95% CI, 0.54–0.98; p = 0.03). Subgroup analysis showed that a maximal neuro-protective effect was reached when HBO was administered immediately after MCAO with an absolute atmospheric pressure (ATA) of 2.0 (50% decrease; 95% CI, 43% -57% decrease; p < 0.0001) and more than 6 hours HBO treatment (53% decrease; 95% CI, 41% -64% decrease; p = 0.0005).

Conclusions

HBO had a neuro-protective effect and improved survival in animal models of MCAO, especially in animals given more than 6 hours of HBO and when given immediately after MCAO with 2.0 ATA.
Introduction

Acute stroke remains a serious issue worldwide [1], especially in low-to-middle-income countries [2]. Cerebral hypoxia causes immediate and secondary cell damage, leading disability or even death. Hyperbaric oxygen therapy (HBO) increases oxygen supply to ischemic tissues and reduces irreversible tissue damage. However, the use of HBO for the treatment of ischemic stroke in the clinic remains controversial [3].

During HBO therapy, a patient breathes 100% oxygen in a pressurized environment of at least 1.4 absolute atmospheres (ATA) [4]. A typical treatment consists of oxygen given at 1.5 to 3.0 ATA for 90 to 120 minutes [5]. The results of recent clinical trials have indicated that HBO might improve neurological outcome in post stroke patients, and that this improvement might be seen even when HBO is administered during chronic late stages [6, 7]. However, other studies have reported no beneficial effects following HBO therapy in post stroke patients [8]. This conflict has been mirrored in the results reported by studies examining HBO in animal models of stroke [9, 10]. The conflict in the literature may be the result of study specific variations in initiation time, treatment duration and HBO pressure.

To address the controversy surrounding the efficacy of HBO for cerebral ischemia treatment, and to investigate variations in HBO efficacy under different experimental conditions, we performed a systematic review and meta-analysis of studies that investigated HBO therapy in animal models of middle cerebral artery occlusion (MCAO).

Materials and Methods

Database and Literature Search Strategies

A systematic search of the literature from January 1960 to September 2015 was conducted using PubMed, Embase, Medline (OvidSP) and Web of Science. The keywords used for the search were “hyperoxia” OR “hyperbaric oxygen” OR “HBO” AND “isch(a)emia” OR “focal cerebral ischemia” OR “stroke” OR “infarct” OR “middle cerebral artery occlusion (MCAO).”

Study selection and inclusion criteria

Studies were independently screened by two investigators (Yang Xu and Ruili Wei). Study inclusion criteria were: 1) HBO was used to treat ischemic stroke induced by transient MCAO, embolic MCAO or permanent MCAO; 2) Study design included a control group; and 3) Neuro-behavior scores, infarct size and mortality were compared between groups. Study exclusion criteria were: 1) Not a primary study; 2) Not an animal study; 3) Not a study of the disease of interest (MCAO); and 4) HBO therapy was not used. A third investigator (Bo Yin) resolved any disagreements between the primary screening investigators. The primary endpoints examined were infarct area or volume (as determined by 2, 3, 5-triphenyltetrazolium chloride (TTC) staining of tissue sections or cross-sectional MRI imaging) and neuro-behavior scores.

Study characteristics

The quality of the evidence in each study was assessed using the following criteria [11]: A) publication after peer review, B) inclusion of a statement concerning temperature control, C) random allocation to treatment or control groups, D) masked induction of ischemia, E) masked assessment of outcome, F) use of anesthetic without significant intrinsic neuroprotective activity, G) use of an appropriate animal model (aged, diabetic or hypertensive), H) appropriate sample size calculation, I) compliance with animal welfare regulations and J) the presence of an appropriate statement of potential conflict of interests. The maximum total score for any study was ten points.
Data Extraction

Data was independently extracted by two investigators according to the inclusion criteria described above (Fig 1). Comparisons were performed between the HBO treatment and control groups of the selected studies. For each comparison, the mean outcome, standard deviation and number of animals in each group were examined. When data was found to be missing, the original authors of the study in question were contacted for additional information. If data were only represented graphically, numerical values were requested from the original authors. If no response was received for numerical data, digital scale software Image J (National Institutes of Health, Bethesda, MD, USA) was utilized to extract numerical data from the available graphical data. We used the ‘#’ symbol to mark these articles, and they were presented in Table 1. Effect size was defined as the improvement in infarct size, neurologic score or combined score in treated animals when compared with the control groups. Other data collected included the timing of treatment initiation (preconditioning before ischemia, treatment concurrent with an ischemic event or treatment after ischemia and reperfusion), duration of HBO, oxygen pressure, ischemia induction method, combined drug therapy and animal morality rate.

Statistical Analysis

For primary outcome comparisons (infarct volumes and neuro-behavior scores), the mean outcomes of treatment groups and the standard deviations of treatment and control groups were expressed as a proportion of control group outcomes. The proportion of the mean values of the HBO groups to the mean values of the control groups was used as a new parameter. Data analysis was conducted using weighted mean differences and a random effects model. To explore the impact of various parameters on estimates of effect size, we performed a stratified meta-analysis using the following parameters: timing of treatment initiation, treatment duration, ischemia induction method, oxygen pressure, combined drug therapy, continuous or intermittent HBO treatment and species of animal used. $p < 0.01$ was considered significant for...
### Table 1. Characteristics of the animal studies included in the meta-analysis.

| Author          | Year | Species | Stroke model | Time of ischemia | Time from onset | HBO treatment | Length | Outcome Measures | Quality score |
|-----------------|------|---------|--------------|------------------|-----------------|---------------|--------|------------------|---------------|
| W. J. Yan #12   | 2011 | SD      | tMCAO        | 120 min          | 5 d before occlusion | 100% O₂ 2.5 ATA | 1 h*5  | Infarct Volume   | 5             |
| Kuppers-Tiedt #13| 2011 | Wistar  | eMCAO        |                  | 45 min or 120 min after occlusion | 100% O₂ 2.4 ATA | 1 h   | Combined         | 6             |
| Mu #14          | 2013 | SD      | pMCAO        | 48 h after occlusion | 100% O₂ 2.5 ATA | 1 h*10        | Infarct Volume | 6             |
| Badr #10        | 2001 | SD      | tMCAO        | 120 min          | 3 h, 6 h, 12h, 23h after occlusion | 100% O₂ 3 ATA | 1 h   | Combined         | 5             |
| Beynon #15      | 2007 | Wistar  | tMCAO        | 150 min          | 90 min or 120 min after occlusion | 100% O₂ 3 ATA | 1 h   | Combined         | 6             |
| Wang #16        | 2012 | SD      | tMCAO        | 60 min           | 3 h before occlusion or 3 h after reperfusion | 100% O₂ 3 ATA | 1 h*1 | Combined         | 4             |
| Veltkamp #17    | 2000 | Wistar  | tMCAO        | 60 min           | Immediately after occlusion | 100% O₂ 1.5 ATA or 100% O₂ 2.5 ATA | 2 h*1 | Infarct Volume   | 6             |
| Lu #9           | 2014 | Wistar  | tMCAO        | 90 min           | 3 h after reperfusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume   | 5             |
| Bian #18        | 2015 | SD      | tMCAO        | 120 min          | 5 d before occlusion | 100% O₂ 2.5 ATA | 1 h*5 | Combined         | 6             |
| Soejima #19     | 2012 | 2H      | tMCAO        | 120 min          | 5 d before occlusion | 100% O₂ 2.5 ATA | 1 h*5 | Combined         | 6             |
| Soejima #20     | 2013 | SD      | tMCAO        | 90 min           | 5 d before occlusion | 100% O₂ 2.5 ATA | 1 h*5 | Combined         | 6             |
| Li #21          | 2009 | SD      | tMCAO        | 90 min           | 4 d before occlusion | 100% O₂ 2.5 ATA | 1 h*4 | Combined         | 6             |
| Chang #22       | 2000 | SD      | tMCAO        | 60 min           | Immediately or 1 h after occlusion | 100% O₂ 3 ATA | 1.5 h*2 | Infarct Volume | 6             |
| Prass #23       | 2000 | SV129   | tMCAO        | 60 min           | 5 d before occlusion | 100% O₂ 3 ATA | 1 h*5 | Infarct Volume   | 5             |
| Qin #24         | 2007 | SD      | tMCAO        | 60 min           | 0.5 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Combined         | 6             |
| Yin #25         | 2003 | SD      | tMCAO        | 120 min          | 6 h after occlusion | 100% O₂ 2.5 ATA | 2 h*1 | Infarct Volume   | 5             |
| Lou #26         | 2006 | SD      | tMCAO        | 90 min           | 1.5 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume   | 6             |
| Li #27          | 2008 | SD      | pMCAO        | 24 h before occlusion | 100% O₂ 2.5 ATA | 2.5 h*1 | Infarct Volume | 3             |
| Gu #28          | 2008 | SD      | pMCAO        | 5 d before occlusion | 100% O₂ 2 ATA | 1 h*5 | Behavioral Score | 5             |
| Schabitz #29    | 2004 | Wistar  | pMCAO        | 2 h after occlusion | 100% O₂ 2 ATA | 1 h*1 | Combined         | 5             |
| Eschenfelder #30| 2008 | SD      | tMCAO        | 90 min           | 1.5 h after reperfusion | 100% O₂ 2 ATA, 2.5 ATA or 3 ATA | 1.5 h*1 | Combined         | 6             |
| Miljkovic-Lolic #31| 2003 | SD      | tMCAO        | 60 min           | Immediately or 1 h before occlusion | 100% O₂ 3 ATA | 1 h*1 | Combined         | 5             |
| Sun #32         | 2014 | C57     | tMCAO        | 90 min           | 15 min after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume   | 5             |
| Sun #33         | 2011 | C57     | tMCAO        | 90 min           | 15 min after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume   | 6             |
| Veltkamp #34    | 2006 | C57     | tMCAO        | 90 min           | 45 min after occlusion or 1 d, 3 d, 5 d after reperfusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume   | 6             |
| Rink #35        | 2010 | Wistar  | tMCAO        | 90 min           | Immediately after occlusion or reperfusion | 100% O₂ 2 ATA | 1.5 h*1 | Infarct Volume | 5             |
| Gunther #36     | 2005 | 2H      | pMCAO        | 0.5 h to 6 h after occlusion | 100% O₂ 2.5 ATA | 1.5 h*2 or 1.5 h*4 | Infarct Volume | 6             |

(Continued)
| Author | Year | Species | Stroke model | Time of ischemia | Time from onset | HBO treatment | Length | Outcome Measures | Quality score |
|--------|------|---------|--------------|-----------------|----------------|---------------|--------|-----------------|---------------|
| Chen # [37] | 2014 | SD | tMCAO | 90 min | 1.5 h after reperfusion | 100% O₂ 2 ATA | 1 h*2, 1 h*4, 1 h*6, or 1 h*8 | Infarct Volume | 6 |
| Weinstein [38] | 1987 | Cat | tMCAO | 6 h | 1 h, 3 h or 4 h after occlusion | 100% O₂ 2.5 ATA | 0.75 h*1 | Infarct Volume | 3 |
| Yan # [39] | 2013 | SD | tMCAO | 120 min | 5 d before occlusion | 100% O₂ 2.5 ATA | 1 h*5 | Infarct Volume | 4 |
| Liu [40] | 2010 | SD | tMCAO | 90 min | 1.5 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume | 6 |
| Jiang [41] | 2006 | SD | tMCAO | - | - | - | - | Infarct Volume | 3 |
| Hou [42] | 2007 | SD | tMCAO | 120 min | 0.5 h after occlusion or 1.5 h after reperfusion | 100% O₂ 2 ATA | 1 h*1 | Infarct Volume | 5 |
| Huang [43] | 2007 | SD | eMCAO | 1 h after occlusion | 100% O₂ 2.5 ATA | 2 h*1 | Combined | 5 |
| Kawamura [44] | 1990 | SD | tMCAO | 4 h | 4 h after occlusion | - | 3.5 h*1 | Infarct Volume | 4 |
| Lou [45] | 2004 | SD | tMCAO or pMCAO | 1.5 h | 4 h, 6h or 12 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume | 6 |
| Yin [46] | 2005 | SD | tMCAO | 2 h | 6 h or 24 h after reperfusion | 100% O₂ 2.5 ATA | 2 h*7 or 2 h*6 | Infarct Volume | 5 |
| Xue # [47] | 2008 | SD | tMCAO | 1.5 h | 3 h after occlusion | 100% O₂ 2 ATA | 9 h*1 or 18 h*1 | Infarct Volume | 5 |
| Xiong [48] | 2000 | SD | tMCAO | 1.5 h | 5 d before occlusion | 100% O₂ 2.5 ATA | 1 h*3 or 1 h*5 | Combined | 5 |
| Sunami [49] | 2000 | SD | tMCAO | 2 h | 10 min after occlusion | 100% O₂ 3 ATA | 2 h*1 | Infarct Volume | 5 |
| Harman [50] | 2012 | SD | pMCAO | - | before occlusion | 100% O₂ 2.8 ATA | 0.75 h*1 | Infarct Volume | 4 |
| Acka [51] | 2007 | Wistar | tMCAO | 1.5h | Immediately after occlusion | 100% O₂ 2.5 ATA | 1.5 h*1 | Infarct Volume | 6 |
| Yin # [52] | 2002 | SD | tMCAO | 2 h | 8 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume | 5 |
| Badr # [53] | 2001 | SD | tMCAO | 2 h | 8 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume | 4 |
| Veltkamp [54] | 2005 | Wistar | tMCAO | 2 h | 0.5 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume | 4 |
| Li [55] | 2008 | SD | tMCAO | 2 h | 1 d before occlusion | 100% O₂ 2.5 ATA | 1 h*4 | Behavioral Score | 5 |
| Veltkamp [56] | 2005 | Wistar | tMCAO | 2 h | 0.5 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Infarct Volume | 5 |
| Sun [57] | 2008 | C57 | tMCAO | 2 h | 0.5 h after occlusion | 100% O₂ 3 ATA | 1.5 h*1 | Infarct Volume | 6 |
| Lou [58] | 2007 | SD | pMCAO | 1 h after occlusion | 100% O₂ 3 ATA | 1 h*1 | Combined | 6 |
| Henninger [59] | 2006 | Wistar | eMCAO | 3 h after occlusion | 100% O₂ 2.5 ATA | 1 h*1 | Infarct Volume | 6 |
| Yang [60] | 2010 | SD | tMCAO | 1h | Immediately after occlusion | 100% O₂ 2.8 ATA | 0.25 h*4 | Infarct Volume | 5 |

# showed the study in which Image J (NIH) software was used to extract data from images.
* means HBO treatment duration and frequency

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multiple comparisons. Forest Plots and funnel plots were generated using RevMan 5.3.5 software (Cochrane Library, London, UK).

Results
Study Inclusion and Characteristics

We identified 51 publications describing the effect of HBO in focal cerebral ischemia that met our inclusion criteria (Table 1). These publications included 47 full articles and 4 abstracts. The earliest study included was published in 1987. Among these studies, 111 comparisons were identified describing outcomes in 2456 animals (all included animals were male). Rat MCAO models (Wistar or Sprague-Dawley rats) were used in 42 studies, 5 studies used mice (C57 or SV129), 3 studies used hyperglycemia or hypertension rats (2H) rats and 1 study was performed in cats. Forty of these studies utilized a transient MCAO (tMCAO) animal model, seven used permanent MCAO (pMCAO), three used Endothelin-1 or autologous blood clot embolic MCAO (eMCAO) and one used both tMCAO and pMCAO. Thirty-five publications reported infarct volume and 14 reported neuro-behavior scores with infarct volume. Two publications only reported neuro-behavior outcomes. Thirteen publications provided morality rate. None of the included studies addressed side effects. Treatment initiation ranged from five days before to two days after ischemia induction. Effect size was measured at a median of 24 hours (6 hours to 28 days) after ischemia onset.

Efficacy

HBO treatment decreased infarct volume by 32% (95% confidence interval (CI), 28%–37% decrease; \( p < 0.00001 \)) with significant heterogeneity (\( \chi^2 = 3345.29, df = 110, p < 0.00001, I^2 = 97\%; \) Fig 2A). The mortality was 18.4% in the HBO group and 26.7% in the control group (Risk Ratio (RR) 0.72, 95% CI, 0.54–0.98, \( p = 0.03 \); Fig 2B).

Subgroup analysis

Treatment initiation ranged from five days before to two days after the onset of ischemia. All treatment initiation subtypes (preconditioning before ischemia, concurrent, post treatment after ischemia and reperfusion) exhibited protective effects (Fig 3A). The smallest infarct size and best neurologic function was observed when HBO was administered immediately after MCAO (Fig 3B). However, HBO reduced infarct volume even when it was initiated 12 hours after ischemia.

Significant neuro-protective effects were observed at all oxygen pressures used in the selected studies (pressures ranged from 1.5 ATA to 3.0 ATA; Fig 3C). However, the maximum neuro-protective effect was observed near 2.0 ATA (50% decrease; 95% CI; range, 43% to 57% decrease; \( \chi^2 = 23.01; df = 3; p < 0.0001 \)).

Protective effects were found for all treatment durations in the selected studies (ranging from 45 minutes to 18 hours), with a maximum effect following approximately 6 hours of HBO treatment (53% decrease; 95% CI; range, 64% decrease to 41% increase; \( \chi^2 = 19.51; df = 4; p = 0.0006 \); Fig 3D).

No statistically significant difference was observed between HBO treatment alone and HBO treatment given in combination with any drug in the selected studies (Fig 4A). Repetitive oxygen therapy trended towards greater efficacy when compared with single oxygen therapy; however, this trend was not significant (\( \chi^2 = 3.90, df = 1, p = 0.05 \); Fig 4B).

Different types of MCAO (transient or permanent) had little effect on evaluation results (\( \chi^2 = 5.38, df = 2, p = 0.05 \); Fig 5A). Subgroup analysis showed HBO effects on different animal
species might not be the same. The reduced infarct volumes in the SD and cat groups were more significant than the volume reductions observed in the other groups, and were minimal in the 2H group ($\chi^2 = 27.88, df = 5, p < 0.0001$; Fig 5B).

Study Quality and Publication Bias

None of the included studies reported statements concerning potential conflicts of interest, sample size calculation or masked induction of ischemia. Thirty-eight of the included studies used random allocation of animals to treatment groups. Only two studies used animal models relevant to specific clinical situations (e.g., hyperglycemia or hypertension) [18, 36]. The
median included quality score was 5 (range 3 to 6). Although no significant differences were observed when studies were ranked by quality score (Fig 5C), this still represents an important potential source of bias. The funnel plot revealed an asymmetrical distribution of studies around the line of identity (S1 Fig).

**Discussion**

This meta-analysis revealed that HBO therapy provoked a significant neuro-protective effect and improved survival in animal models of MCAO. Maximum efficacy was achieved when intervention was implemented immediately after brain ischemia/reperfusion and maintained for more than 6 hours at 2.0 ATA.

The MCAO model is frequently used to imitate acute stroke. Ischemic stroke is induced by arterial occlusion or arterial stenosis. During ischemia, decreased cerebral blood flow reduces the availability of oxygen and glucose, leading to excitatory toxicity, depolarization, inflammation, and the production of nitric oxide (NO), reactive oxygen species and toxic prostanoids [61]. An ischemic core with a surrounding penumbra is formed when the blood flow to surrounding regions is impaired [62]. This is a temporal phenomenon and rarely seen in the clinic. The current review observed that HBO was effective even when HBO was administered 12
hours after the onset of ischemia. These results indicate that the therapeutic window could be expanded. This is a particularly striking result because it indicates that the currently accepted time window for thrombolysis in the clinic of 3 to 6 hours might be too conservative [63]. All HBO treatment dosages were associated with improved neurological function, however, treatment under 2.0 ATA maximized efficacy in animal studies, which is slightly lower than the 2.5 ATA pressure recommended by the Committee of the Undersea and Hyperbaric Medical Society [14]. The current meta-analysis observes that HBO efficacy improvement begins after 3 hours of treatment and reaches a maximum at 6 hours. This pattern of treatment response might be related to decreased oxygen intoxication and improved HBO tolerance. This hypothesis is supported by a study by Beynon et. al. that reported that HBO efficacy appears to be time-dependent [15]. The current meta-analysis observed no additional efficacy when HBO therapy was combined with other drugs. However, combining HBO with thrombolysis was associated with a trend towards fewer hemorrhagic complications [13]. No efficacy differences were observed between ischemia period and the different MCAO variations (eMCAO, pMCAO or tMCAO).

The mechanism of HBO therapy provoked neuroprotection remains unclear. However, reduced brain edema and improved post-ischemia metabolism seem contribute to the observed beneficial effects. HBO appears to increase the expression of antioxidant enzymes, alleviate oxidative stress [55], reduce the formation of hydroxyl radicals [60], enhance superoxide dismutase and catalase activity [55, 64] and inhibit the activity of nitrogen monoxide synthase (NOS) [65]. Additionally, HBO modulate inflammation in the brain by increasing anti-inflammatory cytokines (such as IL-10) and inhibiting pro-inflammatory cytokines (such as TNF-α and IL-1-β) [37]. Furthermore, HBO might reduce interactions between inflammatory chemokines and infiltrating neutrophils, resulting in restricted leukocyte aggregation in the ischemic-hypoxic area. Moreover, HBO may directly affect cyclooxygenase-2 (COX-2) activity in ischemic hemispheres [52]. Finally, it is likely that inhibition of apoptosis by HBO treatment suppresses mitochondrial apoptotic pathways, reduces cytoplasm cytochrome-c levels, decreases caspase enzyme activity and dysregulates the ratio of B cell lymphoma 2 (Bcl-2) and Bcl-2 associated X (Bax) expression, thereby preventing DNA fragmentation [21, 25].

Interestingly, HBO efficacy might be influenced by the animal species. Subgroup analysis indicated HBO was least effective in the 2H group when compared with other species. This reduction in efficacy might be due to the pathological changes caused by hyperglycemia and hypertension in the 2H group. Because most stroke patients have comorbidities, the 2H group may be the best representation of clinical reality among the animal models examined here. Unfortunately, these confounding factors increase the uncertainty of HBO efficacy.

The beneficial effects of HBO therapy observed in animal models were different from those reported in clinical trials. There may be a number of explanations for these discrepancies. First, patients with ischemic stroke might have a narrow therapeutic time window while this time window is relatively longer in animal experiments. Many clinical patients are treated later after stroke onset and showed no beneficial effects [8, 66, 67]. Second, higher than optimal pressure was used for HBO treatment in the studies examined. Although evidence suggests 2.5 ATA may be optimal for improved outcome and smaller infarctions, we consider 2.0 ATA more appropriate in current meta-analysis. Indeed, Sánchez et al. used 1.8 ATA HBO over 6 hours to treat stroke and reported a significant reduction of infarct volume [5]. A clinical trial by Imai et al. reported that HBO therapy at 2.0 ATA has been associated with favorable outcomes [68]. Higher pressure HBO exposure is a subject of concern because high pressures may impair blood-brain barrier integrity and induce free radical production and lipid peroxidation [32, 69–71]. The production of hydroxyl radicals may lead to decreased cerebral blood flow and central nervous system oxygen toxicity [72]. Additionally, interrupted autophagy flux might
play a role. As a clearance pathway, autophagy degrades dysfunctional proteins after MCAO. Interruption of autophagy could result in dysfunctional protein aggregation and neuronal death [9]. Therefore, it is important to consider key autophagy factors (including autophagy-related molecules (Microtubule-associated protein 1 light chain 3, LC3) and autophagy substrates when investigating potentially harmful treatment routines. Additionally, peroxidation lipid (Malonaldehyde, MDA) and NOX levels can help determine optimal HBO pressures in animal experiments.

Limitations
This study has a number of limitations that should be considered. First, the study had a high degree of heterogeneity and included a number of different animal models, therapeutic strategies and end points. Second, publication bias might overestimate the efficacy of HBO therapy because it is difficult to publish negative results. Third, subgroup analysis (with its lack of statistical power) is always risky. Fourth, most of these studies were performed in healthy animals, which may not accurately replicate the conditions of clinical stroke patients with morbidity. Fifth, side effects were not studied in these experiments. Sixth, the software facilitated data extraction used for 9 articles might have added unintended bias; however, an independent analysis comparing extracted and original data demonstrated similar HBO effects. Finally, the results of these studies must be interpreted with care because the results of animal studies may not always be clinically relevant.

Conclusion
This study reports that HBO therapy is associated with a substantial neuroprotective effect and improved survival in animal models of MCAO. This effect was most pronounced when 2.0 ATA HBO therapy was administered for more than 6 hours immediately after MCAO. The oxygen pressure, initiation and length of treatment described in this study should be taken into account to maximize effectiveness when designing future clinical studies.

Supporting Information
S1 PRISMA Checklist. PRISMA checklist. (DOC)
S1 Fig. Funnel plot overseeing publication bias of included studies. (TIF)

Author Contributions
Conceived and designed the experiments: BL RW. Performed the experiments: YX. Analyzed the data: YX RW BY RJ. Contributed reagents/materials/analysis tools: FH. Wrote the paper: YX.

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