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ORIGINAL ARTICLE

# Hyperbaric oxygen for experimental intracerebral haemorrhage: Systematic review and stratified meta-analysis

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## ABSTRACT

**Objective:** Hyperbaric oxygen (HBO) is widely used in treating various neurological diseases. However, HBO for treatment of intracerebral haemorrhage (ICH) remains controversial, in either animal or clinical studies. Therefore, we conducted this systematic review and meta-analysis on studies describing the efficacy of HBO in animal models of ICH.

**Methods:** Studies were identified by searching mainstream databases through November 2015. The efficacy of HBO in animal models of ICH was assessed by changes in the brain water content (BWC), neurobehavioural outcome (NO) or both. Subgroup analyses were performed according to different design characteristics.

**Results:** In total 15 studies met our inclusion criteria. HBO can reduce the BWC (−0.982, 95% CI, −1.148 to −0.817;  $P < 0.01$ ; 57 comparisons), and improve NO (−0.767, 95% CI, −1.376 to −0.159;  $P < 0.01$ ; eight comparisons). HBO was most effective in reducing BWC when given 72 h after ICH for a 4- to 5-day consecutive treatment at the chamber pressure of 3.0 atmosphere absolute. Efficacy was higher with phenobarbital anaesthesia, the blood infusion model and in rabbits.

**Conclusion:** Although HBO was found to be effective in experimental ICH, additional confirmation is needed due to possible publication bias, poor study quality and the limited number of studies conducting clinical trials.

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meta-analysis

## Introduction

Intracerebral haemorrhage (ICH) is the second most common subtype of stroke, with the highest mortality and morbidity rate [1]. Due to unsatisfactory therapeutic effectiveness, the burden of ICH is increasing worldwide, particularly in middle- and low-income countries [2,3]. Therefore, it is necessary to explore new strategies against ICH. One of the most devastating pathophysiological changes following ICH is the formation of perihæmatomal oedema, which is closely related to the severity of underlying bleeding and the mass effect of blood clots that contribute to early neurological deterioration [4,5]. Therefore, reduction of posthaemorrhagic oedema will be a potential target for ICH treatment.

Hyperbaric oxygen (HBO) therapy is a method to inhale 100 per cent oxygen inside a hyperbaric chamber that is pressurized to greater than 1 atm [6]. It has been widely used in treating multiple neurological diseases, including carbon monoxide poisoning, global and focal cerebral ischaemia, vegetative states and cerebral vasospasm after subarachnoid haemorrhage [6–8]. Decreased brain oedema and improved neurological outcomes have been reported in animals treated with HBO after ischaemia [8–10]. The underlying neuroprotective mechanism may involve the improvement of brain

metabolism, reduction of blood–brain barrier permeability, decrease of intracranial pressure, attenuation of inflammatory response and prevention of apoptotic cell death [6,11]. However, ICH has received far less research attention than ischaemic stroke has [12]. Moreover, the limited evidence obtained from animal studies on the efficacy of HBO against ICH remains controversial [13–15]. Therefore, we have conducted this systematic review of animal studies to investigate the neuroprotective properties of HBO on brain oedema (brain water content, BWC) and neurological outcomes after experimental ICH. The BWC and neurological outcomes were chosen as the most relevant indicators for the purpose of this review, as brain oedema is the most devastating and life-threatening complication of ICH [16], and as several studies have shown that the degree of brain oedema surrounding the haematoma is associated with a poor outcome [17–19]. After the brain was harvested, the basal ganglia was separated and weighted on an electronic analytical balance to determine wet weight. Then the sample was heated for 24 h at 100°C in a gravity and weight for dried weight. The percentage of BWC was then calculated according to the following formula: (wet weight—dry weight)/wet weight × 100%. The main purpose of this review is trying to identify key factors, such as the influence of a different time window of treatment, therapeutic

pressure and course on the efficacy of HBO therapy on experimental ICH animals. Related characteristics of study design that may influence the results will also be investigated by stratified meta-analysis. Such preclinical studies will help inform the design of future clinical trials aiming at assessing the neuroprotective potential of HBO treatment following ICH.

## Methods

### Study identification

Bibliographical databases for literature search about experimentally controlled studies of the effect of HBO on BWC and neurological outcome included PubMed, Web of Knowledge, Embase, China National Knowledge Infrastructure, VIP Database for Chinese Technical Periodicals and Wanfang Database (all until October 2014). Additional publications were identified from reference lists of all identified publications and non-systematic reviews. The following search terms were used: intracerebral h(a)emorrhage OR ICH OR intracranial h(a)emorrhage OR h(a)emorrhagic stroke OR stroke AND Hyperbaric oxygen OR HBO(T) OR oxygen therapy OR hyperbaric oxygenation (treating) OR high-pressure oxygen. Studies could be included if they meet all of the following criteria: (I) Experimental intracerebral haemorrhage was induced. (II) HBO was given before or after the induction of ICH. (III) The intervention for control group was room pressure. (IV) The primary and secondary indicators were BWC and neurological deficit, respectively (Figure 1).

### Data extraction

Two authors (Han-jin Cui and Han-yu He) independently extracted data from included studies on methodological quality score, animal species, number, gender, intervention (pressure

of HBO treatment, timing relevant to induction of ICH), ICH induction method, anaesthetic technique used during the operation, efficacy assessment methods and treatment outcomes in treatment and control groups. Disagreements were dealt with by discussion with a third reviewer (Jie-kun Luo). A comparison was defined as an assessment of outcome in treatment groups and control groups after HBO treatment starting at a stated time before or after the onset of ICH. For the comparison, the data for mean outcome, standard deviation (SD) and the number of animals per group were extracted. If included studies used multiple groups, for example, to assess pressure–response relationships or time windows, then the data from each group were extracted individually for analysis. If neurological tests were performed at different time points, only the final test was included. If neurological deficit was assessed by more than one neurological domain, data were combined using meta-analysis for an overall estimate of effect magnitude and standard error. Occasionally, numerical data were not reported in the text, the data were requested from the authors, and if a response was not received, the data were extracted from enlarged, photocopied figures by data analysis software (OriginPro 9.0; OriginLab Corporation, Northampton, MA, USA).

The methodological quality of each study was assessed by using the 8-point Stroke Therapy Academic Industry Roundtable (1999) rating system [20–22]. One point was given for each of the following criteria: presence of randomization, assessment of dose–response relationship, assessment of optimal time window, monitor of physiological parameters, blinded outcome assessment, assessment of at least two outcomes, acute-phase outcome assessment (1–3 days) and chronic-phase outcome assessment (7–30 days). Studies that scored <4 points were considered to be of ‘poor methodological quality’, and studies that scored  $\geq 4$  points were considered to be of ‘good methodological quality’.

### Data analysis

The data analysis was carried out by Hanjin Cui, Haoyu He and Jiekun Luo by using a statistical software package (Stata, version 11.0; StataCorp LP, College Station, TX, USA). The effect of HBO on the total BWC and neurological score were compared between the treatment groups and control groups, using the standardized mean difference (the difference in the effect of HBO between the treatment and control groups was divided by the total SD). This allows comparisons being able to be made if different methods of measurement or different animal species were used. Estimates were pooled by using the DerSimonian and Laird random effects model [23], which is more conservative than a fixed-effect model and is more rational to take into account any statistical heterogeneity found among studies.

We performed a stratified meta-analysis to assess the impact on pre- or post-treatment of HBO, HBO pressure, time and course of administration, study quality score, method of ICH induction, species of animals used and type of anaesthetic used. Publication bias was assessed by visually examining a funnel plot of precision (reciprocal of standard error) against the standardized mean difference, then

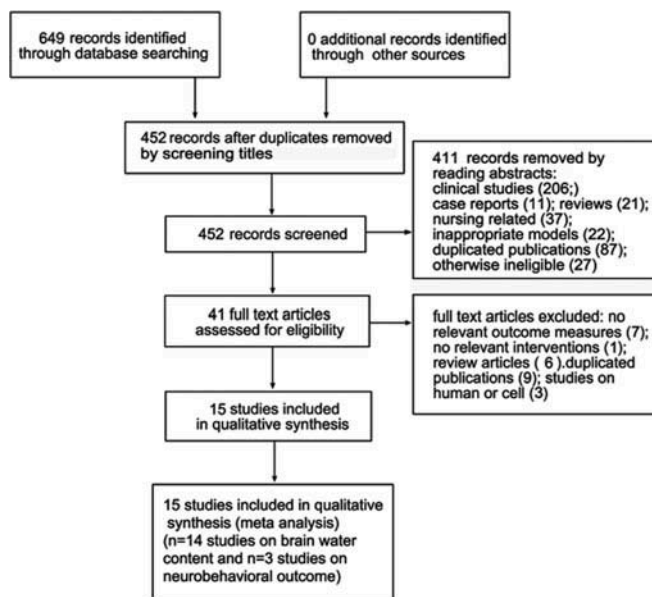


Figure 1. Progression from literature search to meta-analysis. The number of exclusions from the initial literature search is shown.

asymmetry was formally assessed by using the Egger's test (STATA function 'metabias' function of Stata software) [24]. At the same time, we used trim-and-fill method to estimate a summary effect size after adjusting asymmetric funnel plots. Statistical significance was set at  $P < 0.05$ , and the 95% confidence intervals (CIs) of all results were calculated.

## Results

### Methodological designs

The literature search identified 649 potential studies, although most had to be excluded for reasons given in Figure 1. The characteristics of the remaining 15 studies are listed in Table 1. All of these studies report the effect of HBO on BWC and/or neurological outcome after ICH.

The overall study characteristics were shown in Table 1. We extracted the data from these 15 studies, which describe the BWC in 14 studies (65 comparisons) and the neurobehavioural scores in three studies (8 comparisons) (Figure 2). Among them, nine studies employed autologous whole blood model, while four studies applied collagenase model. Only two studies [15,25] tested the efficacy of HBO in both models. Most studies involved Sprague Dawley rat ( $n = 13$ ) with only two exceptions: these two studies were done by using C57 mice ( $n = 1$ ) [15] and rabbits ( $n = 1$ ). Most studies used male animals ( $n = 11$ ), 3 studies [26,27] used both male and female animals and one study [28] did not mention the gender of animals. Only five studies reported the age of animal.

All studies compared the effect of HBO versus room pressure. For six pre-treatments, ICH was induced 24 h after the last HBO treatment. The post-ICH treatment ( $n = 9$ ) of HBO ranged from 1 to 72 h after the induction of ICH. The course of HBO treatment ranged from 1 to 28 days. The chamber pressure of HBO was performed from 1 to 3 atmosphere absolute (ATA).

### Study quality

Of the 15 included, the median of study quality score was four (IQR, 3–4), among them five (33.3%) included studies were regarded as poor methodological quality (three point) studies. Only 1 (6.7%) investigated the pressure–response relationship [29], 12 (80%) reported random allocation of animals to treatment and control groups, two (13.3%) investigated the optimal time window of the treatment, seven (46.7%) monitored the animals' physiological parameters during the induction of ICH, none of these studies assessed the outcome blindly, 15 (100%) assessed at least two acute-phase outcomes and six (40%) assessed chronic-phase outcomes (Table 2).

For the assessment of BWC, the median numbers of animals in the control and treatment groups were six (interquartile range [IQR], 6–8). For evaluation of neurobehavioural outcomes, the median numbers of animals in the control and treatment groups were eight (IQR, 7.5–9).

### Global estimates of efficacy

The global estimate of efficacy of HBO in reducing the BWC was  $-0.982$  (95% CI,  $-1.148$  to  $-0.817$ ;  $P < 0.01$ ; 57 comparisons) (Figure 2A). There is only slight heterogeneity among studies reporting BWC ( $I^2 = 35.8\%$ ). The global estimate of efficacy of HBO in improving the neurobehavioural outcome was  $-0.767$  (95% CI,  $-1.376$  to  $-0.159$ ;  $P < 0.01$ ; 8 comparisons) (Figure 2B). The heterogeneity among comparisons of neurobehavioural outcomes was statistically significant ( $I^2 = 63.6\%$ ).

### Publication bias

Visual inspection of the funnel plots suggested substantial publication bias for both the BWC and neurobehavioural outcomes. The presence of publication bias was supported by Egger's regression results. The trim-and-fill method predicted two theoretically missing comparisons of both BWC and neurobehavioural scores (Figure 3).

### Study characteristics

After stratification of the data according to the study quality score, the highest effect size was found in 4-point studies (effect size,  $-1.070$ ; 95% CI,  $-1.282$  to  $-0.858$ ;  $P < 0.001$ ) followed by 3-point and 5-point studies that showed no apparent beneficial effect (Figure 4A).

The studies used phenobarbital anaesthesia during the induction of ICH exhibited the higher effect size than studies using other anaesthetics did (effect size,  $-1.135$ ; 95% CI,  $-1.466$  to  $-0.805$ ;  $P < 0.001$ ) (Figure 4B).

Our comparative studies using different ICH induction methods detected a higher effect in autologous blood infusion model (effect size,  $-1.040$ ; 95% CI,  $-1.249$  to  $-0.823$ ;  $P < 0.001$ ) than those in collagenase model (Figure 4C).

Timing of HBO treatment ranged from 7 days prior to and 72 h after the induction of ICH. As for 57 unique cohorts of animals, 40.35% of studies administered the intervention at the same time, which was the most common time point, 24 h. HBO treatment most effectively reduced the BWC when given 72 h after ICH (effect size,  $-3.129$ ; 95% CI,  $-4.475$  to  $-1.782$ ;  $P < 0.001$ ), followed by 48 h (effect size,  $-2.231$ ; 95% CI,  $-3.370$  to  $-1.091$ ;  $P < 0.001$ ) after ICH. The pre-treatment at 7 d to 5 d gave the third highest estimate of effect size (effect size,  $-1.549$ ; 95% CI,  $-1.932$  to  $-1.166$ ;  $P < 0.001$ ) (Figure 4D).

We also analysed the pressure–response relationship of HBO and found that the median pressure tested was 2 ATA (IQR, 1–3 ATA). There was a trend for effect size to be higher along with the increase of the pressure. HBO appears to be the most effective when pressure is at 3.0 ATA (effect size,  $-1.163$ ; 95% CI,  $-1.408$  to  $-0.918$ ;  $P < 0.001$ ) (Figure 4E).

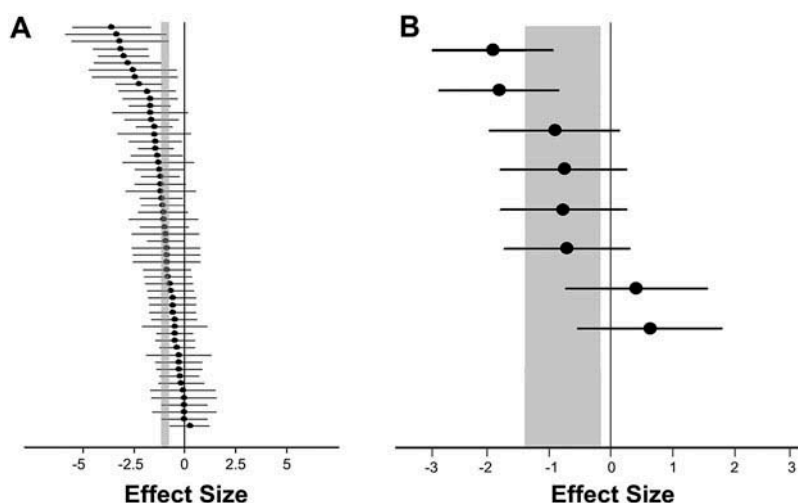
The median course of HBO treatment was 4 days (3–5 days). Studies, in which a 4- to 5-day treatment course was performed, showed the highest effect size (effect size,  $-1.434$ ; 95% CI,  $-1.734$  to  $-1.133$ ;  $P < 0.001$ ) (Figure 4F).

Among all species tested in these studies, HBO showed most beneficial in treatment of rabbits (effect size,  $-1.566$ ; 95% CI,  $-2.188$  to  $-0.943$ ;  $P < 0.001$ ) (Figure 4G).

Table 1. Design characteristics of included studies.

First author, year of publication	Animal (HBO/Con), n	Sex	Age	Method of ICH	Anaesthetic	Intervention (HBO/Con)	Assessment
Xie Q, 2004	SDR(11/11)	M	NR	VII collagenase	CH	0.25 MPa, for 1 h daily for 5 d, started time was not reported/NBO	BWC 5 d after ICH
Qin Z, 2007	SDR(24/6)	M	NR	Whole blood	PS	3.0 ATA for 1 h daily for 2, 3, 5 days before ICH/NBO	BWC 1, 3 d after ICH
Qin Z, 2008a	SDR(10/10)	M	NR	Whole blood	PS	3.0 ATA for 1 h for 1 and 3 d, started 1 h after ICH/NBO	BWC 1, 3 d after ICH
Qin Z, 2008b	SDR(16/16)	M	NR	Whole blood	PS	3.0 ATA for 1 h daily for 5 days before ICH/NBO	BWC 1, 3 d after ICH
Pan G, (unpublished Master thesis, 2010)	Rbt(30/30)	M/ NR	NR	Whole blood	CH	0.2 MPa, 1 h daily for 7 d, started 24, 48, 72 h after ICH/NBO	BWC 7 d after ICH
Shi Z, 2010	SDR(12/12)	M	N	Whole blood	PS	3.0 ATA for 1 h daily for 5 days 24 h before ICH/NBO	BWC 72 h after ICH
Tong X, 2010	SDR(30/30)	M/ F	N	VII collagenase	CH	0.20 MPa for 1 h daily for 4, 7, 14, 21, 28 d, started time was not reported/NBO	BWC and NO 4, 7, 14, 21, 28 d after ICH
Pan X, (unpublished Master thesis, 2011)	SDR(18/6)	M	N	Whole blood	CH	3.0 ATA for 1 h daily for 3, 5, 7 days 24 h before ICH/NBO	BWC 24 or 72 h after ICH
Fang J, 2012	SDR(30/30)	M	NR	Whole blood or VII collagenase	CH	0.1 MPa for 1 h daily for 1, 2, 3, 5 or 7 d, started 24 h after ICH/NBO	BWC 1, 2, 3, 5, 7 d after ICH
Wang A, 2012	SDR(20/20)	M/ F	NR	Whole blood	CH	0.1 MPa for 45 min daily for 6 h, 1, 3, 7, 14 d, started 3 h after ICH/NBO	BWC 6 h, 1, 3, 7, 14 d after ICH
Chen Y, 2013	SDR(72/18)	M	NR	VII collagenase	CH	1.0, 1.8, 2.0, 2.2 ATA for 1 h daily for 1, 3, 5 d, started 24 h after ICH/NBO	BWC 1, 3, 5 d after ICH
Hu H, 2014	SDR(12/12)	M	NR	Whole blood	PS	3.0ATA for 1 h daily for 5 days 24 h before ICH/NBO	BWC and NO 3 d after ICH
Peng Z, 2014	SDR(10/10)	NR	NR	VII collagenase	CH	0.20 MPa for 1 h, started 3 h after ICH/NBO	NO 4, 7, 14, 21, 28 d after ICH
Shi Z, 2014	SDR(12/12)	M	N	Whole blood	PS	3.0 ATA for 1 h daily for 5 days 24 h before ICH/NBO	BWC 72 h after ICH
Zhou W, 2014	C57(36/36)	M	N	Whole blood or VII collagenase	Halothane	3.0 ATA for 1 h, started 0.5, 1, 2 h after ICH/NBO	BWC and NO 3 d after ICH

HBO, hyperbaric oxygen; Con, Control; SDR, Sprague Dawley rats; Rbt: rabbit; CM, C57 mice; M, males; F, female; A, aged; N, normal adult; PS: pentobarbital sodium; CH: chloral hydrate; ATA: atmosphere absolute (10.2 ATA = 1 MPa); NBO: normobaric oxygen; NO, neurological outcome; BWC, brain water content; NR, not reported.



**Figure 2.** Effect sizes of included comparisons. A forest plot of the effect sizes for each comparison measuring (A) brain water content and (B) neurobehavioural outcome. Grey bars represent 95% confidence intervals.

## Discussion

### Summary of evidence

As the first systematic review of HBO for experimental ICH, the present outcomes of this review showed that HBO leads to a substantial reduction in brain oedema and significant improvement in neurological outcomes in intracerebral haemorrhagic animal models. HBO may be a promising therapeutic candidate strategy for ICH. Despite these positive findings, it is premature to make a conclusion that the HBO is exactly effective for the treatment of experimental ICH.

### Study design

The present review showed that HBO was most beneficial in reduction of brain oedema when given 72 h after onset of ICH for a 4- to 5-day consecutive treatment at the chamber

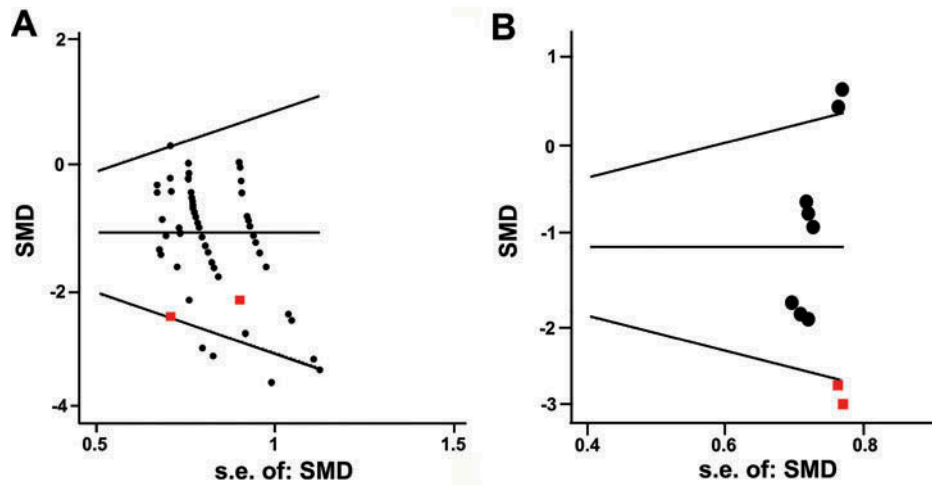
pressure of 3.0 ATA. The main mechanism of HBO on ICH is to reduce brain oedema by means of reducing vascular permeability, enhancing blood–barrier integrity and decreasing the deformability of the red blood cells [14,30]. Therefore, the therapeutic window of ICH treatment should correspond to the time of brain oedema formation, peaking approximately 3 to 4 days after haemorrhaging begins [31]. HBO has been shown to lead to increased levels of free radicals [32] and lowered cerebral blood flow even in normal tissue. This process might cause an inverse steal with an increase in blood flow from brain [33]. Those adverse effects may worsen the brain injury. Thus, the lower effect size generated during an earlier exposure to HBO (prior to 24 h) and/or a longer treatment course (more than 7 days) can be interpreted. Interestingly, 5- to 7-day HBO pre-treatment also showed apparent efficacy. In an ischaemia/perfusion study, ischaemic rats pre-treated through HBO had much better neurological outcomes [34]. Although the underlying therapeutic mechanisms of HBO pre- and post-ICH might be different, the result implies that HBO can be a potential preventive strategy for people with high risk of stroke. Some ischaemic model studies found the neuroprotective effect of HBO at the chamber pressure of 3 ATA [35–37], but lacked pressure–response investigation in comparison with other pressure levels. So it is difficult to say that 3 ATA is the optimal level. The standard protocol for HBO in human clinical use is 2.0–2.4 ATA [38]. Moreover, the finding that prolonged exposure to HBO (>3 ATA) leads to toxicity to central nervous system, which indicates that the level of 3 ATA is a critical point. [39,40].

The most effective result was shown in studies that induced ICH under phenobarbital anaesthesia. This result is in keeping with the findings of Malcolm R Macleod et al. [41] and Hanna M Vesterinen et al. [42]. One study detected that mice suffering from neonatal stroke experienced better neurological outcome after treatment with low-dose phenobarbital (30 mg/kg) [43]. In another study on hypoxic–ischaemic encephalopathy, phenobarbital increased the neuroprotective efficacy of

**Table 2.** Quality characteristics of included studies.

First Author, year of publication	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	Score
Xie Q, 2004[62]		√				√	√		3
Qin Z, 2007[13]				√		√	√		3
Qin Z, 2008a[14]				√		√	√		3
Qin Z, 2008b[30]				√		√	√		3
Pan G, (unpublished Master thesis, 2010)		√	√			√		√	4
Shi Z, 2010[63]		√		√		√	√		4
Tong X, 2010[26]		√		√		√		√	3
Pan X, (unpublished Master thesis, 2011)		√		√		√	√		4
Fang J, 2012[25]		√				√	√	√	4
Wang A, 2012[27]		√				√	√	√	4
Chen Y, 2013[29]	√	√				√	√		4
Hu H, 2014[64]		√		√		√	√		4
Peng Z, 2014[28]		√				√	√	√	4
Shi Z, 2014[65]		√		√		√	√		4
Zhou W, 2014[15]		√	√			√	√	√	5

(1) The dose/response relationship that was investigated, (2) randomization of the experiment, (3) optimal time window of the treatment investigated, (4) monitoring of physiological parameters, (5) blinded outcome assessment, (6) assessment of at least 2 outcomes, (7) outcome assessment in the acute phase (1–3 days) and (8) outcome assessment in the chronic phase (7–30 days).



**Figure 3.** Publication bias. (A, B) Funnel plots. Red squares represent theoretically missing comparisons identified using the trim-and-fill method. SMD: standardized mean difference.

therapeutic hypothermia [44]. These findings might confound the interpretation of the efficacy of HBO under the circumstance of phenobarbital use.

With respect to the ICH induction method, a better efficacy of HBO was found in studies, in which the autologous blood infusion model was induced. Unlike the collagenase model, in which initial bleeding could be seen as early as 10 min and the expansion of haematoma was observed at 1 and 4 h after injection [45], the haematoma of the blood model usually starts to form soon after injection and remains stable in the first 4 h [46]. It is known that the secondary brain injury is mainly caused by haematoma by releasing thrombin, erythrocyte lysis and iron. As a result, the subsequent brain oedema, neurological deficit and recovery process of blood infusion animals will begin several hours earlier than in animals with collagenase injection. Moreover, haematoma size becomes larger and diffuses from the haemorrhage site into the parenchyma in the collagenase model, though its initial volume is similar to the blood model, in which haematoma resolves more quickly [47]. It is because those collagenases are a group of metalloproteinases that degrade interstitial and basement membrane collagen [48]. As well, an obvious drawback of the collagenase model is the enhancement of inflammation [45], which will cause more severe oedema than the blood infusion model does. Finally, disruption of the blood–brain barrier caused by collagenase injection was significantly more serious in comparison with that caused by the blood model. These factors may help explain the better efficacy of HBO in the blood model.

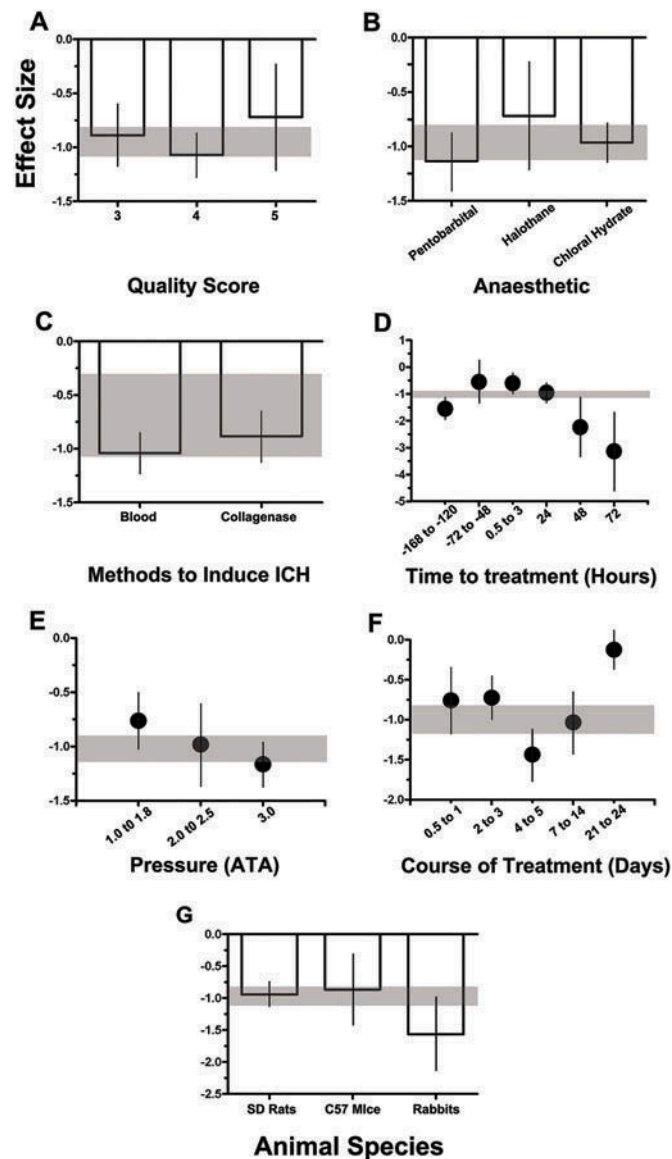
Another finding of this review is that the efficacy of HBO was higher in rabbits than in other murines. Rabbits are the largest small animal model, and are more sensitive in physiologic responses to acute brain injury than their smaller rodent counterparts [49]. But primate studies were lacking in the included review, which would have given a better indication of potential efficacy in human stroke and provide clinical studies with a more useful guide [41].

### Limitations

This systematic review suggests that HBO may be beneficial for managing ICH. However, the present systematic review does have some limitations and the results generated in this review should be interpreted with caution.

First, it is worth noting that the methodological quality of one-third included studies was generally poor (3 points). There are some methodological weaknesses in the included studies. Randomization is required to avoid selection bias. There are three studies, however, that did not resort to random allocation of animals to experimental groups. Blinding is necessary to prevent outcomes from being effected by observer bias. However, none of these studies assessed the outcome blindly. In addition, investigation of the pressure–response relationship and optimal time window of the HBO treatment, assessment of both acute-phase outcomes and chronic-phase outcomes and monitoring the animals' physiological parameters during the induction of ICH are essential factors for a strictly designed study and convincing results. However, none of the included studies conducted these factors at the same time. More high-quality studies are needed to confirm the exact efficacy of HBO on ICH treatment.

Secondly, in this review, the stratified analysis only focuses on the effect of HBO treatment on brain oedema after stroke, due to insufficient data regarding neurological deficit: only three studies using different assessment methods (eight comparisons) examined the functional benefits of HBO treatment. In addition, the heterogeneity of is high (>50%) after the data combination. Thus, current evidence is insufficient to support the efficacy of HBO for ICH in terms of improvement of neurological deficit. Moreover, the assessment of only brain oedema reduction is of limited value in interpreting whether HBO treatment is beneficial or not. Although the haematoma influence on BWC measurement in ICH model is inevitable, the comparison was made between ICH and ICH + HBO group and the effect of haematoma was counteracted. We found that six included studies compared BWC in basal ganglia and cortex,



**Figure 4.** Impact of study design characteristics. Effects of the (A) quality score, (B) anaesthetic used during the induction of ICH, (C) methods of intracerebral haemorrhage induction, (D) time of HBO treatment, (E) pressure, (F) the course of treatment, and (G) animal species are measured as the reduction in brain water content. Error bars represent 95% confidence intervals. The horizontal grey bar represents the global estimate of efficacy for brain water content and its 95% confidence interval.

respectively, while others used basal ganglia and cortex as a whole. However, cortex BWC showed no significant difference between ICH and HBO groups since cerebral oedema is local and presents in the vicinity of a focal oedema-producing lesion usually. Occasionally, oedema spares the adjacent grey matter [50]. Therefore, using basal ganglia and cortex as a whole in BWC measurement is more acceptable. Meanwhile, we noticed that the heterogeneity among comparisons of neurobehavioural outcomes was high with an  $I^2$  value of 63.6%. It might be mainly caused by variability in the animal species, time windows, chamber pressure, ICH induction methods, anaesthetics, observed end points and neurological testing. These factors are difficult to control, especially under circumstance that few studies observed neurological outcome. Performing stratified meta-analysis

according to variety of methodological quality score, anaesthetic technique, ICH induction methods, time to treatment, chamber pressure, course and animal species further reduced the sample size. For example, only one study used rabbit. Although the result showed that HBO is more effective in rabbit model, it does not count as a definite conclusion. Because trials with inadequate sample sizes often run the risk of overestimating intervention benefits [51].

Thirdly, the analysis includes only published studies, plus two unpublished master theses. Negative or neutral studies are less likely to be published, so the results of meta-analysis may be overstated. In fact, Egger's asymmetry test identifies that publication bias does exist and the trim-and-fill approach discovered two studies on BWC that are theoretically missing due to their negative or neutral results. Consequently, the



benefits of HBO on treating brain oedema might have been overestimated. Additionally, unpublished studies will limit useful information on the effect of treatment within certain protocol aspects such as chamber pressure or time of administration.

All included studies that this review examines did not have co-morbid and aged animal models, this generates the fourth limitation in this review, and thus may weaken the predictive value of included studies for clinical trials. Comorbidities can affect efficacy in animal models [52]. Several systematic reviews detected a lower effect size in comorbid animals, compared with healthy animals [41,53]. Evidence from case-control and cohort studies has confirmed that hypertension is the most important single risk factor for ICH, [54,55] and about 80% patients with ICH have a history of hypertension [56]. Diabetes is another proven risk factor for ICH and the relative risk of ICH in patients with diabetes is 1.6-fold in comparison with patients without diabetes [57]. Meanwhile, a consensus is reached through numerous studies that the incidence of intracerebral haemorrhage increases remarkably with age. People aged 85 years and over have an almost 10-fold increase in yearly risk of intracerebral haemorrhage, compared with people aged 45–54 years [58]. Another problem is about the genders of experimental animals. Ignoring animal genders may lead to overestimated data, because oestrogens are believed to provide females with endogenous protection against cerebrovascular events. And abundant evidences are found to support the neuroprotective function of oestrogens in case of cerebral injury. [59,60].

There is a consensus that haemorrhagic diseases are contraindication of HBO, which may increase the risk of re-bleeding in the acute-phase following ICH. Absence of observing re-bleeding and side effects of HBO treatment in animal models of ICH present the fifth limitation of this review. Complications like hyper-excitability of neural networks, aural barotrauma, impaired vision and claustrophobia were reported in clinical trials in other diseases treated with HBO [61]. Because, the clinical trials on HBO against ICH can seldom be found in databases, related complications are sometimes really hard to identify. Therefore, the evidence from present systematic review is insufficient to recommend the routine use of HBO for ICH. To determine these complications may become one of future tasks, either experimentally or clinically.

Finally, although experimental studies have shown that HBO treatment, given before or after ICH, may have neuroprotective effects, no clinical study, at present, has investigated the effects of HBO after haemorrhagic stroke. Animal studies can be fundamental in determining the optimal time window, chamber pressure and course of HBO treatment on ICH. However, these factors should be eventually confirmed by large-scale clinical trials.

Overall, this systematic review on the basis of present available literature showed that HBO is a potentially promising candidate therapeutic strategy for ICH. HBO showed

highest efficacy in reduction brain oedema when administered 72 h after onset of ICH for a 4- to 5-day consecutive treatment at a chamber pressure of 3.0 ATA. However, these encouraging results were only gained from animal studies. Thus, further confirmation is required by additional clinical studies to shed light on the therapeutic potential of HBO in clinical setting.

### Authors' contributor

J.K.L. and H.J.C. participated in the conception and design of the study. H.J.C., H.Y.H. and J.K.L. carried out the literature search the data extraction. A.L.Y., H.J.Z. and J.K.L. carried out the data analysis. H.J.Z. conducted the second reference screen. J.K.L. and H.J.C. contributed to the preparation of the manuscript. All authors read and approved the final manuscript.

### Disclosure/conflicts of interest

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