Advances in diagnosis and treatment of cerebral arterial gas embolism

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Hyperbaric oxygen does not improve cerebral function when started two or four hours after cerebral arterial gas embolism in swine

Weenink RP, Hollmann MW, Vrijdag XC, van Lienden KP, de Boo DW, Stevens MF, van Gulik TM, van Hulst RA
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Abstract

**Introduction:** Hyperbaric oxygen therapy (HBOT) is the accepted treatment for cerebral arterial gas embolism (CAGE). Although earlier start of HBOT is associated with better outcome, it is unknown how much delay can be tolerated before start of HBOT. This study investigates the effect of HBOT on cerebral function in swine when initiated 2 or 4 h after CAGE.

**Methods:** Under general anesthesia, probes to measure intracranial pressure (ICP), brain oxygen tension (PbtO$_2$), and brain microdialysis, and electrodes for electroencephalography were placed. The electroencephalogram (quantified using temporal brain symmetry index (tBSI)) was suppressed during 1 h by repeated injection of air boluses through a catheter placed in the right ascending pharyngeal artery. HBOT was administered using US Navy Treatment Table 6 after 2 or 4 h delay. Control animals were maintained on an inspiratory oxygen fraction of 0.4.

**Results:** ICP increased to a mean maximum of 19 mmHg (SD 4.5 mmHg) due to the embolization procedure. HBOT significantly increased PbtO$_2$ in both groups treated with HBOT (mean maximum PbtO$_2$ 390 mmHg, SD 177 mmHg). There were no significant differences between groups in regard to tBSI (control vs 2 h delay p=0.078, control vs 4 h delay p=0.150), ICP and microdialysis values.

**Conclusions:** We did not observe an effect of HBOT on cerebral function after a delay of 2 or 4. The injury caused in our model could be too severe for a single session of HBOT to be effective. Our study should not change current HBOT strategies for CAGE but further research is necessary to elucidate our results. Whether less severe injury benefits from HBOT should be investigated in models employing smaller amounts of air and clinical outcome measures.
Introduction

Cerebral arterial gas embolism (CAGE) is a feared complication of invasive medical procedures, for instance cardiac surgery (1). It can also occur in diving when air trapped in the lungs expands during ascent and causes pulmonary barotrauma with subsequent flow of air into the pulmonary venous system and thence to the brain. The generally accepted treatment for CAGE is hyperbaric oxygen therapy (HBOT) which ameliorates injury by decreasing bubble size and providing high partial oxygen pressure to critically perfused cerebral tissue (2). Although HBOT is a relatively safe procedure, the treatment has moderate risks. Problems may occur during transportation of patients to a hyperbaric facility as well as due to the suboptimal clinical care that can be delivered to the patient during HBOT. Furthermore HBOT itself can cause barotrauma, and the high partial oxygen pressures involved carry a risk of cerebral and pulmonary oxygen toxicity (3). Research into the use of HBOT in CAGE is therefore necessary to identify the most optimal treatment strategy in this disease.

One of the unanswered questions in this field is the optimal timing of HBOT. Although earlier start of treatment is associated with better outcome (4), no data are available on the maximum delay that can be tolerated before HBOT must be started. Because of the low incidence of CAGE and its heterogeneous presentation (4), the use of adequate animal models is of great importance in CAGE research (5). We previously published a swine model that employs clinically relevant methods such as intracranial pressure (ICP) and quantitative electroencephalography (qEEG) for assessment of the effects of CAGE on cerebral hemodynamics, metabolism and function (6, 7). In the present article, we report on the effect of HBOT when started 2 or 4 h after induction of CAGE in an adapted version of this model.
Materials and Methods

General handling
After approval of the Animal Ethics Committee of the Academic Medical Center, Amsterdam, The Netherlands and in accordance with European Community guidelines, 22 female Landrace pigs weighing 35-44 kg were used for this study. Animals were premedicated with intramuscular ketamine 15 mg/kg (Eurovet Animal Health, Bladel, The Netherlands), midazolam 2 mg/kg (Actavis, Hafnarfjordur, Iceland) and atropine sulfate 0.01 mg/kg (Pharmachemie, Haarlem, The Netherlands). After intubation, the animals were ventilated (Servo 900C, Siemens, München, Germany) in volume controlled mode with an inspiratory oxygen fraction (FiO₂) of 0.4, frequency 18/min and positive end-expiratory pressure of 4 mmHg. Arterial carbon dioxide tension was maintained between 35 and 40 mmHg by changing minute volume. Anesthesia was continued with intravenous ketamine 10-15 mg/kg/h, sufentanil 5-10 µg/kg/h (Hameln Pharmaceuticals, Hameln, Germany), midazolam 1.5 mg/kg/h and pancuronium bromide 0.15 mg/kg/h (Organon, Oss, The Netherlands). An aluminum emergency blanket was used to maintain normothermia (37-38°C in the swine). Blood pressure was measured invasively through a catheter placed in the brachial artery. A urinary catheter was placed in all animals.

Cerebral catheter, probes and electrodes
Access to the right femoral artery was obtained using the Seldinger technique. Under fluoroscopic guidance a 5F guiding catheter (Guider Softip XF, Boston Scientific, Natick, MA) was advanced to the right common carotid artery. Through this catheter an Ascent Occlusion Balloon Catheter (Johnson & Johnson, New Brunswick, NJ) was positioned in the right ascending pharyngeal artery (the ascending pharyngeal arteries are the most important arteries supplying the pig brain). This balloon catheter allows for air injection distal to the balloon when it is inflated. One calibrated ICP sensor (Codman, Raynham, MA), one Licox temperature probe (Integra, Plainsboro, NJ), two Licox brain oxygen tension (PbtO₂) probes (Integra) and two microdialysis probes (Carnegie Medicine AB, Solna, Sweden) were
positioned in the cerebral tissue as described earlier (8). The Licox temperature probe was necessary to continuously correct PbtO$_2$ for the actual brain temperature. The microdialysis probes were continuously flushed with artificial cerebrospinal fluid (Carnegie Medicine AB) at 1 µL/min. 9 subdermal wire electrodes (Ives EEG Solutions, Newburyport, MA) were placed according to a method adapted from the international 10-20 system as described earlier (7). The EEG signal was recorded and analyzed as described earlier (7). Temporal brain symmetry index (tBSI) was calculated over each 10 s of EEG data and was continuously displayed in the operating room. The tBSI calculates spectral changes in the EEG by comparing the current EEG with a defined normal baseline. It is a normalized parameter within the range [0-1]. A higher tBSI value represents a larger deviation from the baseline EEG (9).

**Embolization and data acquisition**

After a stabilization period of at least 1 h and confirmation of correct positioning of the tip of the balloon catheter by angiography, the baseline as required for tBSI calculations was defined. This was followed by inflation of the balloon in the ascending pharyngeal artery. Balloon inflation did not cause change of the recorded parameters, including EEG signals, in any of the animals. Air embolism was inflicted according to the following protocol. Initially 0.5 ml room air was injected, followed by repeated injection of 0.2, 0.3 or 0.5 ml to reach and maintain a tBSI of at least 0.5. The catheter was flushed with saline between injections. The elevated tBSI was maintained by repeated air injections for 1 h, after which air embolism was stopped and the balloon deflated to restore normal cerebral perfusion.

Animals were randomly assigned to one of three groups. In groups 2HOURS and 4HOURS, HBOT was commenced 2 or 4 h after start of air injection, respectively. 45 min before start of HBOT these animals were transported to the hyperbaric facilities, while connected to a portable ventilator (Pneupac 2R, Smith Medical, St Paul, MN), using FiO$_2$=0.4. End tidal carbon dioxide level was maintained stable by adjusting minute volume. No other manipulations needed to be performed since the transport-cart was the
same as the operating table used during the preparations and all monitoring equipment and intravenous pumps were transported with the pig. Transportation took approximately 10 min, after which the pig arrived in the hyperbaric chamber and was connected to the same type of ventilator and using the same settings as in the preparation phase.

HBOT was administered using a single session of US Navy Treatment Table 6, which is the most commonly used treatment table for CAGE (figure 1). At the end of the treatment FiO$_2$ was switched back to 0.4, and the experiment was terminated 12 min later. Total duration of the experiment after start of air embolism was 7 h (2 h delay plus 4 h 48 min treatment plus 12 min after return to normal atmospheric pressure) in the 2HOURS group and 9 h (4 h delay plus 4 h 48 min treatment plus 12 min after return to normal atmospheric pressure) in the 4HOURS group. Animals in the
CONTROL group did not receive HBOT and were maintained on FiO$_2$=0.4 for 9 h. Investigators could not be blinded for group allocation because of the necessary transportation of the 2HOURS and 4HOURS animals to the hyperbaric facility and the obvious difference between the 2HOURS and 4HOURS group with regard to the elapsed time from start of air embolism to start of HBOT.

Heart rate, blood pressure, body temperature, and ICP were recorded at t=15 min and t=30 min after air embolism and every 30 min thereafter. Blood gas analysis was performed hourly. The EEG was recorded continuously and analyzed offline. Average tBSI and mean amplitude were calculated for the 10 min period around t=15 min, t=30 min, and every 30 min thereafter, as described earlier (7). Vials containing the effluent of the microdialysis probes were changed every 15 min for 2 h after air embolism and every 30 min thereafter. Vials were analyzed for glucose, lactate, glycerol and pyruvate concentrations, which were corrected for recovery rate as determined in a preliminary in vitro experiment (results not displayed). Left and right PbtO$_2$ were recorded every 30 min in all groups, except during HBOT when the values were recorded halfway during each oxygen and each air period. The last PbtO$_2$ values in the HBOT groups were recorded 10 min after return to FiO$_2$=0.4 at normal atmospheric pressure. At the end of the experiments, animals were sacrificed using potassium chloride.

**Statistics**

Preliminary control experiments (not published) resulted in an average tBSI of 0.59 with a standard deviation of 0.1 at the end of the experiments. These numbers and an expected intervention effect of 25% in both HBOT groups resulted in a variance of means of 0.07. Sample size calculation using one way analysis of variance showed that this variance of means could be detected with 80% power at the 0.05 level by using 6 animals per group and 3 groups.

In previous experiments we observed that in some animals the embolization process was complicated by transient massive hypertension, some-
times with tachycardia. During the following hours, this usually led to excessive ICP increase with concurrent decrease in cerebral perfusion pressure and development of an isoelectric EEG. Unfortunately, we have not been able to optimize our model in such a way that these adverse events were completely avoided, and therefore we decided to exclude animals in which ICP≥40 mmHg developed. In these cases the experiment was terminated and the animal was not used for the analysis. Excluded experiments were repeated to maintain group size of n=6.
Figure 3. Temporal brain symmetry index and intracranial pressure in CONTROL versus 2HOURS and CONTROL versus 4HOURS groups. Error bars represent 1 standard deviation. Y-axis crosses x-axis at start of hyperbaric oxygenation. Solid line = experimental groups; dashed line = control group.
tBSI was defined as the primary outcome measure of the study. ICP, brain lactate, brain glycerol and brain lactate/pyruvate ratio were secondary outcomes. For comparison between CONTROL and 2HOURS group, the first 7 h of data from the CONTROL group were used. For comparison between CONTROL and 4HOURS group, the full 9 h of data from the CONTROL group were used. Significance of differences between groups at the same time point was calculated using non-parametric tests for independent samples (Mann-Whitney when comparing two groups, Kruskal-Wallis when comparing three groups); differences between time points within a single group were analyzed using Wilcoxon signed-rank test. Significance

<table>
<thead>
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<th></th>
<th>CO</th>
<th>2H</th>
<th>4H</th>
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<td>injected air volume (ml)</td>
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<td>heart rate (min⁻¹)</td>
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<td>MAP (mmHg)</td>
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Table 1. General and cerebral parameters at the start of the experiment and start and end of group; MAP = mean arterial pressure; L = left; R = right; Br = brain; L/P = lactate/pyruvate; MAMP t=0; a = significant difference between CONTROL, 2HOURS, and 4HOURS groups at t=0; b = significant difference between CONTROL and 4HOURS groups at t=END; c = significant difference between t=START and t=END in this group.
of changes in tBSI were further analyzed using a linear mixed model with group (CONTROL vs 2HOURS or CONTROL vs 4HOURS), time (treated as covariate) and group by time interactions as fixed effects, using a first order autoregressive covariance structure to account for repeated measurements within the same animal. Since we were interested in the effects of HBOT, only the time points from start to end of HBOT were used for the linear mixed models. All tests were two-sided, and statistical significance was accepted at p<0.05. When analyzing differences between PbtO₂ significance was accepted at p<0.008 (Bonferroni correction for six comparisons).
Results

Of the 22 animals, 4 animals reached an ICP≥40 mmHg (3 in the 2HOURS group and 1 in the 4HOURS group) and were thus excluded from further analysis as determined before start of the study. In all these animals, the progressive ICP increase was already evident before start of HBOT. Thus, a total of 18 animals (n=6 per group) were analyzed. Body weight and amount of air injected were not significantly different between the three groups. Table 1 shows the values for general and brain specific values in the three groups at the start of the experiment, start of HBOT, and end of HBOT. Although there was a statistically significant difference in regard to heart rate, brain glucose, and right brain lactate/pyruvate ratio between the three groups at t=0, at the start of HBOT no significant differences between the groups were present. There were no differences between CONTROL and 2HOURS group at the end of HBOT. There were small but significant differences between PaCO₂ and pH between CONTROL and 4HOURS group at the end of HBOT. HBOT resulted in large and significant increase of PbtO₂ during the treatment session (figure 2), while in the control group no clinically relevant changes in PbtO₂ occurred. However, PbtO₂ values in the 2HOURS and 4HOURS group were equal to values in the CONTROL group after return to normal atmospheric pressure and FiO₂=0.4 (table 1). While tBSI in the CONTROL group tended to increase after approximately 2 h after embolization, tBSI in the 2HOURS and 4HOURS group showed a decreasing trend, most notably in the 2HOURS group (figure 3). However, the differences of tBSI at the end of HBOT between groups failed to reach statistical significance (CONTROL vs 2HOURS p=0.078, CONTROL vs 4HOURS p=0.150). Additionally, linear mixed models analysis resulted in non-significant differences between the groups (group by time interaction p=0.197 for 2HOURS vs CONTROL and p=0.597 for 4HOURS vs CONTROL). ICP in all animals showed increase in the first hours after embolization (mean ICP increase 12 mmHg, not significantly different between groups), followed by slight decrease (figure 3). The microdialysis
markers lactate, glycerol, and lactate/pyruvate ratio are displayed in figure 4 (averages and standard deviations are given in table 1, ICP and microdialysis data were not analyzed using linear mixed models).

Discussion

We used an EEG based strategy to inflict CAGE in swine, in order to determine the effect of HBOT after 2 and 4 h delay. Despite the fact that the treated animals were subjected to significantly higher PbtO$_2$ than
the control animals, there were no significant differences in regard to tBSI, ICP, brain lactate, brain glycerol, and brain lactate/pyruvate ratio between groups.

The amount of delay that can be tolerated before commencement of HBOT in CAGE is unknown. In humans only retrospective studies have been performed. Ziser et al. (10) reported on 17 patients with CAGE and found a significant relationship between time to HBOT and outcome. The recent study of Bessereau et al. (4) showed that patients with neurological sequelae in their mixed group of arterial and venous cerebral air embolism were more likely to have received HBOT more than 7 h after injury. In some case reports, recovery after a delay of several days have been reported (11, 12). While many different animal models have been used in CAGE research (13), in all of the studies that included HBOT the therapy was started within 0 to 60 min after induction of CAGE (6, 14-18). Thus, while in the clinical situation a delay of several hours is common, no data are available on the result of these delays on the effectiveness of HBOT. Animal studies on HBOT in non-CAGE transient ischemic stroke suggest that HBOT may be effective up to 6 h after onset of ischemia, but these results have as of yet not been confirmed in human studies (19).

The current study is based on the article by Van Hulst et al. (6) in which a similar animal model was used to demonstrate the effectiveness of HBOT in reducing ICP after a delay of 3 and 60 min after CAGE. However, in this study the embolization resulted in large increases in ICP, which in time would probably not have been compatible with life. Since our goal was to extend the duration of delay after CAGE, we were required to use smaller amounts of damage to the brain than had been used in this previous study. Based on previous research (7, 17, 20, 21) we chose qEEG (specifically tBSI) as the primary outcome measure because of its global character, high temporal resolution, easy applicability in the clinical situation, and demonstrated usefulness in our animal model. By using qEEG we tried to inflict a constant amount of damage in which HBOT would still be expected to be effective. We specifically chose not to inject a fixed
(or weight based) amount of air, since our previous investigations demonstrated that in our model this leads to a wide variation of the effects of the air embolism on the brain (7, 8). We believe this to be caused by the random distribution of the air bubbles through the cerebral vasculature. Our strategy using titrated embolization was based on work of other researchers who demonstrated reproducible injury by titrating embolization based on EEG or somatosensory evoked potentials (14, 16, 22-34).

Further advantages of the current model include the fact that the pig is a large animal and is known for its conformity with human anatomy and physiology (35). The pig brain (albeit much smaller than the human brain) allows the application of human techniques for assessment of cerebral condition, in our model ICP, PbtO₂, microdialysis, and qEEG. This enables comparison of our results with human studies. Furthermore, the specific cerebrovascular anatomy of the pig – with a freely anastomosing network of arterioles at the base of the brain, the rete mirabile – allows for unilateral occlusion of the carotid circulation without alteration of the EEG signal. This allowed us to inflate a balloon in one of the ascending pharyngeal arteries (the equivalents of the human internal carotid arteries) without disturbing cerebral function. By inflating the balloon, we prevented retrograde flow of air into the external carotid circulation, thereby allowing for more selective administration of the air.

Despite the abovementioned efforts and the fact that HBOT resulted in large and significant increase in PbtO₂, we have not been able to demonstrate an effect of HBOT on tBSI and microdialysis values in this study. There are three possible explanations for these results.

Firstly, the failure to demonstrate a significant difference between the groups may be due to type II error. The data on tBSI (figure 3) suggest that there may have been some effect of HBOT on the EEG recordings in our study, although tBSI is already somewhat lower in the intervention groups at the start of HBOT. Therefore, our study could have been underpowered, resulting in failure to detect the difference in tBSI be-
tween groups. The minimum effect size that could be detected with 80% power with the current study setup was 0.66, while the actual effect size observed was only 0.34, mostly due to larger variance of the data. This would suggest that despite our efforts the amount of damage inflicted was not consistent enough to result in predictable changes to the cerebral parameters. Further improvements to our model should therefore focus on more selective methods of embolization.

The second explanation is that there is actually no difference between the groups. A negative effect of delay on the effectiveness of HBOT in CAGE is understandable. Early after induction of CAGE air bubbles are present in the cerebral vessels. The increased atmospheric pressure used in HBOT compresses these bubbles and promotes passage to the venous circulation. Although large bubbles can remain in the arterial vasculature for hours (36), most bubbles introduced in our model will have disappeared during the 2 or 4 h delay. Thrombi may have formed due to prolonged stasis of blood (37). Under these circumstances HBOT may still have an effect, but only because of the hyperoxygenation, immunomodulation and ICP reduction it causes (1). Nevertheless, several reports have suggested beneficial effects of HBOT in CAGE, even after a delay of hours to days (11, 12). This would suggest that the amount of damage inflicted in our model was too severe for HBOT to be effective. Despite the fact that our model did not include progressive ICP increases (these animals were excluded), our embolization resulted in prolonged severe EEG disturbances and an average maximum brain lactate of 4.6 mmol/l. The clinical equivalent of these measurements in our animals are unknown since we did not awake the animals from anesthesia. It is difficult to compare our microdialysis results with human studies, since microdialysis probe characteristics and settings (and therefore recovery rates) vary widely throughout the studies. In general it can be stated that our brain lactate value of 4.6 mmol/l is in line with or even higher than values found in patients with severe traumatic brain injury (38-41). This may indicate that the injury inflicted in our model is too severe for HBOT to be beneficial. On the other hand, previous studies used an embolization protocol in which the
somatosensory evoked potential in cats was decreased to 10% of baseline (16, 22, 23). While no EEG measurements were performed in these animals, it may be expected that the electrochemical disturbances in these animals were even more profound than in our model. Nevertheless, these studies did show a beneficial effect of HBOT in CAGE, possibly because the delay between CAGE and HBOT was only 15 min. In contrast to the clinical situation, where HBOT is usually extended or repeated based on clinical or ancillary examinations, we only provided one session of HBOT. Furthermore, some patients demonstrate improvement during follow up after treatment, while we sacrificed the animals soon after termination of HBOT. It is conceivable that additional treatments with HBOT or extended follow-up would have resulted in beneficial effects, but we believe this to be impracticable from a biotechnical point of view.

A third explanation for our results may be that our outcome parameter tBSI is an inadequate representative of cerebral function and therefore an inappropriate surrogate for human outcome. The effects of CAGE on the cerebrum are multifaceted and not only include infarction leading to electrochemical dysfunction, cerebral edema, and cell death, but also an inflammatory response. We hypothesized that by quantifying brain metabolism, electrical function, and edema we would obtain a general assessment of cerebral status. Unfortunately, we did not perform histological examinations or cerebral imaging nor did we quantify the inflammatory response. Furthermore, several important aspects of outcome, such as cognitive effects of CAGE, were impossible to test in our animal study.

We have not included a control group in which HBOT was started immediately after induction of CAGE. The main reason for not including such a group is the fact that it was practically impossible to perform the experiments in this fashion, since the hyperbaric facilities were only available for the actual treatment sessions and not for the necessary preparations and embolization procedure. Moreover, the effectiveness of direct commencement of HBOT has previously been demonstrated in a model almost identical to ours, in which an even larger amount of cere-
bral injury was inflicted (6).

Only the 2HOURS and 4HOURS animals were transported to the hyperbaric facilities. We undertook great care in preventing any influence of transportation on the animals by minimizing transport time, ventilating the animal with the same FiO$_2$, maintaining end tidal carbon dioxide tension equal to before transportation, and moving all monitoring and other equipment together with the animal. We did not observe any influences of transportation on all measured parameters, although our methodology precludes definitive exclusion of bias due to transportation.

Recommendations on the treatment of CAGE advise prompt administration of 100% oxygen when the diagnosis is suspected (2). In the current study we maintained the animals on FiO$_2$=0.4 until start of HBOT. This was done because in many clinical scenarios the suspected diagnosis of CAGE is delayed for a certain period of time after the insult has occurred (4). This especially occurs in clinical cases of CAGE, where the immediate effects of the embolism may be obscured by general anesthesia. In these cases 100% oxygen will not immediately be given. Secondly, we hypothesized that the beneficial effects of 100% oxygen given during 2 or 4 h after the insult might negate the additional positive effects of subsequent HBOT.

Despite efforts to refine the model by delivering small amounts of air as directly into the carotid cerebral circulation as possible (8) and choosing a highly sensitive primary outcome measure (7), 4 of the 22 animals (18%) experienced progressive ICP increase in the hours following embolization. In three of these animals, the embolization process had been complicated by a period of massive hypertension with tachycardia. In concordance with previous studies (42) we believe these autonomic disorders to be caused by brainstem ischemia, a known issue in CAGE models (43). Although hypertension promotes passage of bubbles through the capillaries in the acute phase of embolization (2), it is known to be detrimental in the following hours since hypertension leads to increased damage to
the blood-brain barrier, which results in more cerebral edema and ICP increase (44). We chose to exclude all animals in which ICP≥40 mmHg developed in order to keep the amount of damage inflicted as consistent as possible.

The most important question is what the current study contributes to the discussion on the effectiveness of HBOT in CAGE. The use of HBOT in this disease is rational from a theoretical point of view, and its effect has been documented in animal studies and retrospective clinical series. Thus, for ethical reasons a placebo controlled trial has never been performed and probably never will be. This makes the development and use of animal models vitally important (5). We are the first to study the effect of delay in HBOT using an animal model, moreover a large animal model that has proven its use in CAGE research. This makes our results interesting and, despite the fact that we recognize that the present study should not change the current treatment strategies for CAGE, asks for more research using even more refined animal models. The use of clinical outcome parameters seems to be of vital importance as we conclude that this is the only way to reliably determine the clinical equivalent of the damage inflicted. Although difficult from a biotechnical point of view, repeat sessions of HBOT or extended observation after treatment may reveal beneficial effects not detectable in this study.

**Conclusions**

In our swine model of CAGE, we were not able to demonstrate improvement in qEEG, ICP and microdialysis values when HBOT was started after a delay of 2 or 4 h. This may be caused by type II error or by the fact that there is actually no effect of HBOT in this situation. If the latter is the case, then the injury inflicted in our model may have been too severe for HBOT to be effective. Further research using clinical outcome measures should be performed in order to answer the question regarding the maximum tolerable delay until start of HBOT in CAGE.
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