Advances in diagnosis and treatment of cerebral arterial gas embolism

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Citation for published version (APA):
Cerebral arterial gas embolism in swine; comparison of two sites for air injection

J Neurosci Methods 2011; 194:336-41
Cerebral arterial gas embolism is a risk in diving and occurs as a complication in surgery and interventional radiology. Swine models for cerebral arterial gas embolism have been used in the past. However, injection of air into the main artery feeding the pig brain – the ascending pharyngeal artery – might be complicated by the presence of the carotid rete, an arteriolar network at the base of the brain. On the other hand, anastomoses between external and internal carotid territories are present in the pig. In order to determine the most appropriate vessel for air injection, we performed experiments in which air was injected into either the ascending pharyngeal artery or the external carotid artery. We injected 0.25 ml/kg of room air selectively into the ascending pharyngeal artery or the external carotid artery of 35-40 kg Landrace pigs (n=8). We assessed the effect on cerebral metabolism by measuring intracranial pressure, brain oxygen tension, and brain glucose and lactate concentrations using cerebral microdialysis. Intracranial pressure and brain oxygen tension changed significantly in both groups, but did not differ between groups. Brain lactate increased significantly more in pigs in which air was injected into the ascending pharyngeal artery. Intracranial pressure, brain oxygen tension, and brain lactate correlated after injection of air into the ascending pharyngeal artery, but not after injection into the external carotid artery. Our model is suitable for investigation of cerebral arterial gas embolism. The ascending pharyngeal artery is the most appropriate vessel for air injection.
Introduction

Cerebral arterial gas embolism (CAGE) can occur in divers when air trapped in the lungs expands during the ascent and causes lung over-distension damage (1, 2). It can also occur as a complication in surgery or interventional radiology. CAGE is a serious disorder often resulting in permanent neurological dysfunction. Current treatment mainly relies on hyperbaric oxygen therapy which ameliorates the injury by decreasing bubble size and increasing oxygenation. Hyperbaric oxygen therapy also attenuates the inflammatory process that follows the passage of bubbles through the vascular bed (3). Most of the clinical evidence on the effectiveness of HBOT in CAGE comes from case reports and retrospective studies including small groups of patients. These reports have been convincing to such an extent that performing prospective trials in humans seems unethical. In order to facilitate further advances in CAGE research, an adequate animal model is needed. Although several other animals such as cats (4), rabbits (5), and dogs (6) have been used in the past, pigs were frequently used in different models of air embolism (7-11). Unfortunately, the application and volume of air and its effects varied over a wide range.

We have developed a porcine model of CAGE, using clinically relevant measurements such as intracranial pressure (ICP), brain oxygen tension (PbtO\textsubscript{2}), and microdialysis to assess the effects of air embolism on cerebral metabolism (12). This model has proven its usefulness in CAGE research (13). We primarily chose the pig because of the similarities between human and swine anatomy and cardiovascular physiology (14). Moreover, the brain of smaller laboratory animals cannot accommodate the probes we require for our measurements.

Since the pig has extensive anastomoses between external and internal carotid territories (15), injection into the external carotid artery (ECA) results in entrance of air into the pig brain. However, the primary vessels supplying the pig brain are the bilateral ascending pharyngeal arteries.
(APA). We were interested if the APA would be a more appropriate vessel for air embolization. Our main concern in this regard was the carotid rete, a network of finely entangled arterioles that forms out of each APA (15). The internal carotid arteries are formed out of these retia. The carotid rete cannot be passed with an angiography catheter, so the air has to be delivered proximal to this structure. The small size of the vessels in the rete might retain all injected air, thus preventing it from entering the cerebral circulation.

In order to assess which of the vessels would be the most appropriate for air embolization experiments, we performed experiments in which we injected equal amounts of air into either the APA or the ECA of the pig. We assessed the effect of air embolism on cerebral metabolism by measuring ICP, PbtO$_2$, and brain glucose and lactate. A larger effect on neuronal metabolism after injection of air in one of the two target vessels would indicate that more air reaches the brain following injection in this particular artery. Furthermore, since the measurements acquired with the brain oxygen and microdialysis probes only represent cerebral metabolism in the immediate vicinity of the probe, we were interested in the degree of correlation between ICP, PbtO$_2$, and brain lactate. A higher degree of correlation after injection in one of the vessels would suggest this vessel as being the most appropriate for our experiments.

**Material and Methods**

**Animal handling**

Approval of this study was obtained from the Animal Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands. Animal care was in accordance with European Union guidelines. Subjects were 16 female 35-40 kg crossbred Landrace pigs. Anaesthesia was induced 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 which the animals were intubated and anaesthesia continued with intravenous ketamine 10-15 mg/kg/h, sufentanil 5-6 µg/kg/h (Hameln Pharmaceuticals, Hameln, Germany), midazolam 1.5 mg/kg/h, and pancuronium bromide 0.1 mg/kg/h (Organon, Oss, The Netherlands). After intubation, animals were connected to a ventilator (Servo Ventilator 900C, Siemens-Elema, Sweden) and ventilated in a volume-controlled mode. Ventilation parameters were: frequency 18/min, inspiratory oxygen fraction 0.4, inspiration time 25%, pause time 10%, positive end-expiratory pressure 4 mmHg. Blood gases were taken hourly and PaCO₂ was maintained at 35-40 mmHg by adjusting minute volume (usually 7.5±0.5 l). Arterial blood pressure (measured by means of a catheter placed in one of the brachial arteries), oxygen saturation, capnography, pulse rate, and rectal temperature were continuously monitored. A bladder catheter was placed in all animals. Body tempera-
ture was maintained at 37-38 °C by means of an aluminum emergency blanket. At the end of the experiment, animals were sacrificed with potassium chloride.

**Arterial catheter**

Access to the right femoral artery was obtained using the Seldinger technique. A 4F angiography catheter (Radiofocus Glidecath, Terumo, Tokyo, Japan) was advanced over a guide wire (Radiofocus, Terumo, Tokyo, Japan) under fluoroscopic guidance using Ultravist 300 (Bayer, Mijdrecht, The Netherlands). The tip of the catheter was placed either in one of the ascending pharyngeal arteries (APA group) or in one of the external carotid arteries (ECA group) (figure 1).

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**Figure 2. Schematic view of the head of the pig after preparation of the burr holes.**
Intracerebral probes
The techniques used have described before (12). In short, after removal of a 2 by 3 cm sized scalp flap centered over the sagittal and coronal sutures, the skull was exposed. Three burr holes were created as depicted in figure 2. After calibration of the probes two Licox (Integra, Plainsboro, NJ, USA) brain oxygen catheter-micro-probes (one in each dorsal burr hole), two CMA 20 Elite (Carnegie Medicine AB, Solna, Sweden) microdialysis probes (one in each dorsal burr hole), one Licox brain temperature catheter-micro-probe (frontal burr hole), and one Codman (Raynham, MA, USA) ICP probe (frontal burr hole) were advanced 15-20 mm through the dura. Left and right PbtO$_2$ were corrected for brain temperature as measured by the temperature probe. The oxygen probes were advanced a few millimeters more into the brain if the recorded value did not increase at least 15 mmHg after increasing the inspired oxygen fraction to 1.0 for 15 min. The microdialysis probes used had a length of 10 mm, a 0.5 mm diameter, and a cut-off value of 20 kDa. The probes were perfused by a CMA 100 microdialysis pump with artificial cerebrospinal fluid (CMA, Carnegie Medicine AB, Solna, Sweden). The vials containing the dialysate were changed every 15 min and analyzed immediately for the amount of glucose and lactate using a CMA 600 analyzer. Values presented here have been corrected for the recovery rate (16) as determined in a preliminary in vitro experiment. Recovery rate was 76 percent for glucose and 89 percent for lactate.

Embolization
A 1 h stabilization period was provided after completion of all surgical procedures, after which 0.25 ml/kg of room air was injected in 60 s through the catheter placed in either the APA or the ECA. ICP and PbtO$_2$ were recorded 30, 60, 90, and 120 min after embolization. Glucose and lactate concentration were measured in the microdialysis vials every 15 min. The animals were sacrificed 120 min after air injection.

Statistical analysis
Data was analyzed using SPSS for Windows software. Values are given
as average ± SD unless stated otherwise. Differences between baseline and 120 min after embolization were calculated using two sided paired t-tests. Differences between groups at a single time point were calculated using two sided non-paired t-tests. Differences between groups for repeated measurements were calculated using the area under the curve. Correlation between parameters was quantified using Spearman’s rho. Statistical significance was accepted at $p<0.05$.

## Results

All animals survived the experimental protocol. Values obtained at baseline and at the end of the experiment are presented in table 1. There were no differences between the groups at $t=0$, except for a small but significant difference between body temperature ($37.1 ^\circ C$ in the APA group versus $37.5 ^\circ C$ in the ECA group) as well as a small but significant

<table>
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<tr>
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<tr>
<td></td>
<td>ascending pharyngeal artery</td>
<td>external carotid artery</td>
<td>ascending pharyngeal artery</td>
<td>external carotid artery</td>
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<tr>
<td>heart rate (min$^{-1}$)</td>
<td>84 ± 21</td>
<td>93 ± 31</td>
<td>91 ± 22</td>
<td>114 ± 34</td>
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<td>MAP (mmHg)</td>
<td>88 ± 17</td>
<td>96 ± 11</td>
<td>77 ± 13</td>
<td>91 ± 18</td>
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<td>body temperature ($^\circ C$)</td>
<td>37,1 ± 0,3$^b$</td>
<td>37,5 ± 0,3$^b$</td>
<td>37 ± 0,4</td>
<td>38 ± 0,4</td>
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<tr>
<td>blood pH</td>
<td>7,46 ± 0,05</td>
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<td>7,47 ± 0,03</td>
<td>7,44 ± 0,03</td>
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<td>PaO$_2$ (mmHg)</td>
<td>234 ± 52</td>
<td>234 ± 23</td>
<td>199 ± 45</td>
<td>210 ± 12$^a$</td>
</tr>
<tr>
<td>PaCO$_2$ (mmHg)</td>
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<td>40 ± 2</td>
<td>39 ± 2</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>ICP (mmHg)</td>
<td>7 ± 2</td>
<td>8 ± 3</td>
<td>25 ± 17$^a$</td>
<td>32 ± 20$^a$</td>
</tr>
<tr>
<td>CPP (mmHg)</td>
<td>81 ± 16</td>
<td>87 ± 13</td>
<td>52 ± 28</td>
<td>67 ± 23$^a$</td>
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<tr>
<td>PbrO$_2$ (mmHg)</td>
<td>22 ± 6</td>
<td>26 ± 5</td>
<td>11 ± 5$^a$</td>
<td>15 ± 10$^a$</td>
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<td>brain glucose (mmol/l)</td>
<td>1,3 ± 0,7</td>
<td>0,6 ± 0,2</td>
<td>0,6 ± 0,3</td>
<td>0,3 ± 0,3</td>
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<tr>
<td>brain lactate (mmol/l)</td>
<td>0,7 ± 0,2</td>
<td>0,5 ± 0,2</td>
<td>2,7 ± 2,0$^{ab}$</td>
<td>1,0 ± 0,5$^b$</td>
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Table 1. Data of variables in both groups (both n=8) at baseline and two hours after embolization. Values are mean ± SD. MAP = mean arterial pressure; ICP = intracranial pressure; CPP = cerebral perfusion pressure; $^a$ = significant difference between baseline and $t=120$; $^b$ = significant difference between groups.
Comparison of two sites for air injection

decrease in arterial oxygen tension during the experiment in the ECA group (234±23 mmHg at t=0, 210±12 mmHg at t=120).

ICP increased significantly in both groups but there was no difference in ICP between groups. Since PbtO₂, brain glucose, and brain lactate did not differ significantly between both hemispheres, the values of both sides of the brain were averaged for each time point. Brain oxygen at t=120 was significantly lower than baseline in both groups, but the difference between the APA and ECA group was not significant. Brain glucose values did not change significantly during the experiments in both groups. There was a significant difference in lactate concentration between groups, the APA group showing a larger increase in lactate (figure 3, p=0.03). We determined correlation of our outcome measures at the end of the experiment (figure 4). There was a significant correlation between ICP, PbtO₂, and brain lactate in the APA group, while no such correlation was found in the ECA group.

Figure 3. Brain lactate concentration ± SEM during two hours after air embolism. Solid line = ascending pharyngeal artery; interrupted line = external carotid artery. Difference is significant (p=0.03).
Discussion

In this study we investigated whether injection of air into the APA or the ECA would be most appropriate for CAGE experiments. The most appropriate vessel was predefined as being the artery that resulted in the largest effects on neuronal metabolism after air injection. Although

Figure 4. Correlation of measured variables at t=120 min. Spearman's rho values shown in each graph. Left column = ascending pharyngeal artery; right column = external carotid artery; ° = p<0.05.
ICP and PbtO$_2$ did not differ between groups, we did observe a larger increase in brain lactate level after embolization of the APA. Furthermore, we observed a significant correlation between ICP, PbtO$_2$, and brain lactate after injection into the APA, which was not the case after embolization of the ECA. Together, these results suggest that use of the APA is more appropriate in our model.

In order to properly investigate the effect of CAGE on the brain, it is essential to inject air into the cerebral vasculature. Although ligation of multiple cerebral vessels would also produce global ischemia (17, 18), the effect of intravascular air on cerebral blood flow and the blood brain barrier as well as the inflammatory response that follows passage of air would not be adequately modeled in this way (19-22). In order to determine which vessel is the most appropriate for injection of air into the pig cerebral vasculature, we measured cerebral function after air embolization into two different arteries. Our main concern was the presence of the carotid rete at the cranial base. The size of the vessels in this carotid rete has been reported to range from 74 µm (23) to 154 µm (24). We hypothesized that air injected proximal of the carotid rete might lodge in these vessels, preventing entrance of air into the circle of Willis. Our results show that this is not the case and that a substantial part of the air passes through the carotid rete into the cerebral vasculature.

Brain glucose and lactate concentrations were measured using cerebral microdialysis. A drawback of this technique is that it only samples the extracellular fluid in an area quite close to the probe (25). The same holds for our measurement of PbtO$_2$. Focal changes might therefore be under- or overestimated due to sampling error. However, there was no correlation between the variables measured after embolization of the ECA, whereas a significant correlation was observed after air injection into the APA. This finding might suggest that air injected into the latter artery disperses more globally through the brain, while embolization of the ECA results in more local distribution of the air.
Brain lactate increased significantly more when air was injected in the APA. This indicates that more air reaches the cerebral vessels when embolization takes place through this artery. We believe this difference can be explained by the fact that when the air is injected in the ECA, it can only reach the circle of Willis through small anastomoses between the ECA and the carotid rete. It would therefore also be expected that the variance of the values obtained would be lower after injection into the APA than after injection into the ECA. We were not able to confirm that in our study. This might be explained by the fact that the cerebral vascular anatomy of the swine does not allow determination of the exact distribution of air within the brain. Variation in cerebral perfusion dynamics between individual pigs might result in different dispersion of air through the vasculature, giving rise to diverse reactions. If, for instance, a large amount of air accumulates in the posterior circulation, a larger cardiovascular response can be expected (26).

The larger increase in brain lactate in the APA group was not associated with a larger increase in ICP or a larger decrease in PbtO$_2$. A recent study in patients with severe traumatic brain injury has shown that lactate increase precedes ICP increase in these patients by more than 2 h (27). This suggests that if the duration of our experiments would have been longer, it might well be possible that the larger increase in brain lactate in the APA groups would be reflected by a larger increase in ICP.

The amount of glucose in brain microdialysate is indicative of the energy available for cerebral metabolism (28). We have previously shown a decrease in brain glucose after CAGE in the pig (12). These results are in line with the findings in the present study, although the decrease in brain glucose we found was not significant and did not differ between groups. Brain lactate increased more in animals embolized via the APA than in those in which the air was injected in the ECA. In the latter group the lactate level seemed to be decreasing by the end of the experiment, while in the former group it increased to levels comparable to those found in patients admitted with severe traumatic brain injury.
Comparison of two sites for air injection

(29). It must be noted that an increase in lactate concentration is not purely indicative of anaerobic metabolism. A state of hypermetabolism as occurs during cellular stress is also accompanied by a rise in lactate (30). In order to differentiate between hypermetabolism and ischemia, one might argue to measure the lactate/pyruvate ratio as an indicator of true ischemia (16). However, it has been demonstrated that brain lactate is a better predictor of ICP increase than lactate/pyruvate ratio in patients with severe traumatic brain injury (27).

There was a baseline difference between groups in regard to body temperature. Furthermore, we observed a significant decrease in arterial oxygen tension during the experiment in the ECA group but not in the APA group. However, since all values remained well within normal limits for the duration of the experiments, we believe it to be unlikely that these differences have influenced our results.

We did not investigate the effect of air injection into the vertebral artery, because the vertebral arteries in pigs are very small and have only little contribution to cerebral perfusion (31). Furthermore, anastomoses between vertebrobasilar and carotid vasculature and thus the carotid rete exist. Finally, injection into the vertebrobasilar system will probably result in brain stem infarction, with subsequent unpredictable and severe hemodynamic changes (26). Although we did observe an increase in blood pressure and heart rate in most of our animals of both groups, these changes were usually moderate and always transient, with values returning to normal within 10 min.

In conclusion, changes in ICP and PbtO$_2$ were comparable between the groups, but brain lactate increased significantly more when the air was injected in the APA. Only embolization of the APA resulted in significant correlation between ICP, PbtO$_2$ and brain lactate. We believe the swine to be a suitable animal for cerebral arterial gas embolism experiments and suggest the ascending pharyngeal artery as the preferred vessel for air injection.
References

Comparison of two sites for air injection