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A rat model for cerebral air microembolisation

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ABSTRACT

Subtle cerebral air microembolisation (CAM) is a typical complication of various medical interventions such as open heart surgery or angiography and can cause transient or permanent neurological and neuropsychological deficits. Evaluation of the underlying pathophysiology requires animal models that allow embolisation of air bubbles of defined diameter and number. Herein we present a method for the production of gas bubbles of defined diameter and their injection into the carotid artery of rats. The number of gas microemboli injected is quantified digitally using a high speed optical image capturing system and a custom-made software.

In a first pilot study, 0, 50, 100, 400 and 800 gas bubbles of 160 μm in diameter were injected into the carotid artery of rats. Offline evaluation revealed a high constancy of the bubble diameters (mean 159.95 ± 9.25 μm, range 144–188 μm) and the number of bubbles injected. First preliminary data indicate that with increasing number of bubbles embolised, more animals revealed neurological deficits and (particularly with higher bubble counts) brain infarctions on TTC-staining. Interestingly, also animals without overt infarcts on TTC-staining displayed neurological deficits in an apparently dose dependent fashion, indicating subtle brain damage by air embolism.

In conclusion, the method presented allows injecting air bubbles of defined number and diameter into cerebral arteries of rats. This technique facilitates animal research in the field of air embolisation.

1. Introduction

Cerebral air microembolisation (CAM) is a typical complication of various medical interventions such as open heart surgery, neurosurgery, diagnostic or therapeutic angiography or central venous catheterization (Heckmann et al., 2000; Gale and Leslie, 2004; Gupta et al., 2007; Gerriets et al., 2010). Small numbers of gaseous microemboli can be found following regular diving or in patients with mechanical heart valves (Kaps et al., 1997; Knauth et al., 1997; Gerriets et al., 2003a,b; Erdem et al., 2009).

It is undisputed that massive CAM (i.e. caused by diving accidents) is a life threatening condition and results in severe and predominantly ischemic damage of the brain and the spinal cord. The biological consequences of small amounts of cerebral air emboli, in contrast, are still a matter of debate. Recent clinical trials suggest that air bubbles may play a pivotal role in the genesis of persistent neuropsychological deficits following open heart surgery or angiography (Gerriets et al., 2010). Moreover, regular exposure to cerebral air microembolism in scuba divers or in patients with artificial heart valves has been suspected to cause cognitive deficits and subclinical white matter lesions in MRI studies (Gerriets et al., 2003a,b; Knauth et al., 1997; Erdem et al., 2009).

Although CAM might affect millions of people worldwide, the underlying pathophysiology of air bubble induced brain damage is largely unknown. The lack of appropriate animal models is one of the most dominant obstacles for dedicated research in this field.

Herein we describe a method for CAM in a rat model that allows embolisation of air bubbles of defined number and diameter into the carotid artery.

2. Materials and methods

2.1. Making of air microbubbles

For the production of the air microbubbles, a custom-made device was developed. A small customized glass capillary

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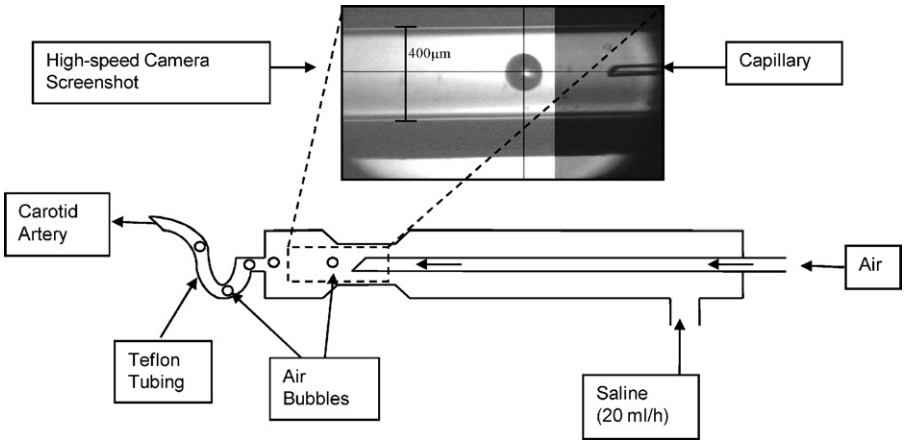

Fig. 1. Setup of bubble generator. Number and size of air bubbles can be determined by variation of air flow, flow velocity of saline, diameter of the capillary (15–40 μm), and the position of the capillary within the outer tube (diameter: 450 μm; see Section 2 for details). Automatic recording and determination of bubble quantity and size is achieved by a high speed optical image capturing system connected to a computer equipped with custom-made software.

(15–40 μm, as needed) was positioned within a larger glass tube (450 μm in diameter, Ringcaps, Hirschmann Laborgeräte GmbH) with a slight taper on the one side (Fig. 1). The outer tube was flooded with saline with a constant flow (20 ml/h) by an infusion pump (MC Medizintechnik GmbH, Alzenau) while air at a precisely adjustable pressure (controlled by a pressure regulator; Watson Smith Ltd., Leeds, England) was piped through the capillary. Pressure range was required from 0.5 to 1.5 bar to vary the number of bubbles produced per minute. The pressure required furthermore depended on the capillary diameter and on the specific mean arterial blood pressure of the rat. The tip of the capillary was positioned in the centre of taper in order to increase saline flow velocity at this point. Air bubbles formed at the tip of the capillary and were carried with the saline flow. The sizes of the bubbles (40–250 μm) could be varied by adjusting the flow rate of the saline within the outer tube. The diameter of the capillary and its position within the taper of the outer tube furthermore affected bubble size.

Careful adjustment of number and size of the air bubbles was performed before the bubble generator was connected to the carotid artery of the rat. Therefore the system was put under hydrostatic pressure (75 mm Hg) to simulate the mean arterial blood pressure of the animal during the adjustment process. Then the saline with the air bubbles was allowed to flow into the carotid artery of the animal by opening a glass valve between the Teflon tubing and the glass tube to switch from the hydrostatic pressure system to the rat.

2.2. Quantification of number and size of air microbubbles

A high speed optical image capturing system (Camera A602f-2, Basler, Switzerland) was focused on the tapering to record the bubble formation (Fig. 1). The system was connected to a personal computer (Mac Book Pro, Mac OS X, version 10.5.1). By using custom-made software, size and number of all air bubbles flowing through the catheter were recorded automatically and displayed.

2.3. Animal preparation

All procedures are in accordance with our institutional guidelines and the German animal protection legislation and were approved by the regional ethics committee (Regierungspraesidium Darmstadt; AZ B2/203). Twenty-five male Wistar rats (Harlan Winkelmann, Borchen, Germany) were anesthetized with 5% isoflurane delivered in air for 2 min within an enclosed chamber. Anesthesia was maintained with 2–3% isoflurane delivered in air at 0.5 l/min via a facial mask during surgery. Spontaneous breathing was maintained. Body temperature was continuously monitored with a rectal probe and maintained at 36.5–37.0 °C with a feed-back controlled heating pad.

The right common carotid (CCA), internal carotid (ICA) and external carotid artery (ECA) were exposed through a midline incision of the neck. The ECA was isolated, and the superior thyroid and the occipital arteries were cauterized. The distal portion of the ECA was ligated with a 5–0 suture and transsected to create an ECA-stump with a length of approximately 5 mm. The pterygopalatine branch of the ICA was also ligated with a 5–0 suture.

2.4. Application of air bubbles to the cerebral circulation

Teflon-50 tubing was deaired, connected with the air bubble generator and inserted into the ECA-stump through an arteriotomy. The tip of the tubing was placed close to the carotid bifurcation in order to allow a free passage of air bubbles into the physiological blood stream from the CCA to the ICA.

2.5. Study design

The rats (body weight 255–326 g; mean 296 ± 22.4 g) were randomised into 5 groups ($n = 5$ each) and received 0, 50, 100, 400 or 800 air microemboli in saline. The bubble generator was adjusted to create air bubbles of 160 μm in diameter. This diameter was chosen as a first attempt, as it lies somewhere in the middle of the diameter range, that can be produced with our device. The infusion was carried out within approximately 2 min at a speed of 20 ml/h, equivalent to an infusion volume of approximately 0.67 ml saline per animal. In order to minimize the total injection volume, the lowest infusion speed was chosen, that did carry the air bubbles appropriately.

After air infusion, the ECA was ligated, the tubing removed and the wounds closed carefully. Then the animals were allowed to recover from anaesthesia.

Three additional animals were likewise subjected to sham embolisation (saline without air bubbles), to determine possible effects of saline infusion into the carotid artery on physiological parameters. In these animals arterial blood pressure was monitored with a PE-50-catheter inserted into the tail artery from 30 min before until 30 min after carotid artery infusion.

Clinical testing was performed at baseline and after 24 h, using a previously described 10 point-score for assessment of motor, coordinative and sensory functions such as contralateral forelimb