Increasing Doses of Intracarotid Air and Cerebral Blood Flow in Rabbits

S.C. Helps, MSc, M. Meyer-Witting, FFARCS, P.L. Reilly, FRACS, MD, and D.F. Gorman, FACOM, PhD

We studied the natural history of brain air embolism by observing bubbles in the pial vessels of rabbits and the effect of different doses of intracarotid air on brain function and blood flow. We identified and then studied two doses of air; 25 μl in five rabbits caused rapid bubble transit, recovery, and then deterioration in brain function and blood flow and 400 μl in five rabbits caused temporary bubble trapping and sustained deterioration in brain function. These dose responses correlate well with the natural history of divers with air embolism of the brain. All doses of air caused both arteriolar dilatation and reduced blood flow, which were independent of dose, whereas the detrimental effect of air embolism on brain function was dose dependent. Our results suggest that this is a good model of brain air embolism. (Stroke 1990;21:1340-1345)

We have studied the natural history of air embolism of the brain by observing bubbles in the pial arteries of rabbits. Most (>80%) bubbles do not block pial blood flow and pass through the arterioles and capillaries to be eventually collected in jugular vein air traps. Similar findings have been reported in other species, and such passage of bubbles may explain the spontaneous recovery seen in both dogs and divers after air embolism of the brain.

Both extremes of air embolism have been identified in our model and reported. Continued infusions of air until bubbles block the exposed pial vessels are lethal. Conversely, after infusion of the smallest dose of intracarotid air that will reliably cause observable bubbles (25 μl), bubbles appear but rapidly pass through the pial vessels. Both local cerebral blood flow (CBF) and brain function measured immediately after this bubble transit are normal. However, CBF and brain function then slowly but progressively deteriorate over the next 90 minutes. Pial vessels respond to bubbles at all doses by dilating, but only a lethal dose of air causes significant brain stem effects, including transient hypertension followed by progressive hypotension, bradycardia, and respiratory depression.

The aim of the studies reported here was to identify and study an intermediate bubble insult, that is, a dose of air that caused bubbles to be trapped temporarily in the pial arteries, but one in which the bubbles eventually cleared and the rabbits survived.

Materials and Methods

We used New Zealand White rabbits (2.1–2.4 kg) as the behavior of pial vessels in this species parallels that of intraparenchymal brain vessels of similar size. This study was performed in accordance with the guidelines and with the approval of the Animal Ethics Committee of the Institute of Medical and Veterinary Science (Adelaide). We anesthetized 41 male rabbits with 2 g/kg i.v. urethane, which is a suitable general anesthetic for studying function in both the central and the peripheral nervous systems. All rabbits were prepared in an identical manner, and rectal temperature was maintained at 38°C with a heating pad.

A tracheostomy was performed. Femoral arterial and venous silicone catheters (602-175, Dow-Corning, Corning, N.Y.) were introduced for sampling of blood, recording of blood pressure, and infusion of fluid (3 ml/kg/hr of lactated Ringer’s solution). The left external and internal carotid arteries were isolated. A silicone cannula was introduced retrogradely into the external carotid artery so that its tip was adjacent to the internal carotid artery, which remained patent. The rabbit was then placed in the sphinx position in a stereotactic frame (David...
FIGURE 1. Typical continuous tracing of cortical somatosensory evoked response signal in rabbits. A: Recording from control rabbit that received saline infusion. Arrow indicates time at which saline was infused. B: Tracing from rabbit treated with 25 μl intracarotid air. Shaded bar indicates time during which air was visible in pial arterioles. C: Tracing from rabbit treated with 400 μl intracarotid air. Shaded bar indicates time during which air was visible in pial arterioles.

Kopf Instruments, Tujunga, Calif.), connected to a ventilator (Harvard Apparatus, South Natick, Mass.), and paralyzed with 10 mg/kg/hr gallamine as a continuous infusion. The physiologic ranges of Paco2 and Pao2 were maintained by varying the ventilator stroke volume and rate and the concentration of inspired oxygen. The scalp was reflected, and a craniotomy was performed over the left sensorimotor cortex. The dura was removed, and a 1-cm-diameter polypropylene cylinder was cemented to the skull. This cylinder was filled with paraffin oil to a depth of 1 cm to maintain pial surface pH. A 1-mm burr hole was made over the right sensorimotor cortex.

To measure CBF, electrodes were prepared from sharpened 0.2-mm Teflon-coated platinum wire. The bare platinum tips were placed in the right and left cerebral cortices to a depth of 1 mm using an operating microscope (OPMI, Carl Zeiss Pty. Ltd., Adelaide, South Australia) and microelectrode carriers. An indifferent electrode of silver-silver chloride wire was placed subcutaneously in the rabbit's back. A two-channel polarographic amplifier system was used to measure hydrogen concentration, and hydrogen gas (approximately 10% by volume) was added to the ventilator inlet for 5 minutes. The first 30 seconds of the subsequent hydrogen clearance curve was ignored, and CBF (as milliliters per minute per 100 g) was calculated from the next 90 seconds of the curve using the initial slope index method. This method was chosen because it is regional, highly reproducible and repeatable and does not affect CBF itself. The initial slope index gives an estimate of the fastest blood flow within the tissue being sampled such that blood flow decrements after air embolism will be underestimated and not overestimated.

Two minutes after the beginning of each hydrogen clearance, 50 μl blood was taken for analysis of Pao2 and PaCO2. Simultaneously, rectal temperature, mean arterial blood pressure (MABP), and heart rate were recorded and the brain surface was photographed (FP4 film, Ilford, Melbourne, Australia). An arteriole with an external diameter of 40-100 μm was selected, and the external diameter of a chosen fixed point of the vessel was measured from the projected film. Arteriolar diameter was calibrated against a 35-μm suture. These vessels were chosen because if bubbles become trapped, it is in arterioles of this size.

A silver ball electrode 0.75 mm in diameter was placed on the exposed brain surface to measure cortical somatosensory evoked response. The rabbit's right forepaw was stimulated via needle electrodes for 0.5 msec at a frequency of 1 Hz and a voltage three times that producing a detectable response. The ball electrode was positioned to record the maximum signal from the left somatosensory area 1, with a frequency response of 5–2,000 Hz. A continuous recording of the cortical signal was displayed on a chart recorder (Neotrace NEO400ZEF, Neomedix Systems, Sydney, Australia) (Figure 1A). In addition, 2 minutes after the beginning of each hydrogen clearance, 80 discrete evoked responses were recorded and averaged. In pilot studies, the second positive wave (P2) of the cortical response was found to be the most sensitive to air embolism (Figure 2).

In preliminary studies using 21 rabbits, bubble transit times (seconds from the appearance of the first bubble until all bubbles are cleared from the exposed pial vessels) were determined for 50 (n=4), 100 (n=5), 200 (n=4), 300 (n=3), and 400 (n=5) μl.
air injected into the left internal carotid arteries of rabbits. All doses caused pial air embolism, but only in rabbits given 400 μl air were bubbles trapped (stationary) in the pial vessels (transit time range 30–1,740 seconds). Consequently, the experimental rabbits in the second phase of the study were given 400 μl air.

In the second phase, 15 rabbits were maintained within the physiologic ranges of Pao2 and Paco2 for at least 90 minutes. After that time, either 400 μl air and 200 μl saline (400 μl embolism group, n=5) or 200 μl saline alone (control group, n=10) were injected into the carotid artery cannula at a rate of 400 μl/15 seconds. Both groups were monitored for 3 hours following the infusion and then killed by a barbiturate overdose. All parameters were recorded 2 and 15 minutes after the insult and then every 15 minutes. The 400 μl embolism and control groups were also compared with identically prepared rabbits into which we had injected 25 μl air (25 μl embolism group, n=5). Results from this group have been reported.

For each parameter, the mean of the preinfusion data was assigned a value of 100%. All subsequent data were recorded as a percentage of the preinfusion mean. Data were tested by analyses of variance, regression analyses, and t tests. A significance level of p<0.05 was chosen, and when simultaneous multiple comparisons were performed Bonferroni's method was used. Results are given as mean±SEM.

Results

The MABP, Paco2, PaO2, and heart rate did not change significantly at any time in these rabbits. Similarly, there were no significant changes in CBF, cortical somatosensory evoked response, or arteriole diameter before the infusion.

Following infusion of 400 μl (160–190 μl/kg) air into the left internal carotid artery, bubbles appeared in the pial arteries of all five rabbits within 10 seconds. These bubbles were initially trapped in pial arterioles 50–200 μm in diameter but were eventually displaced by blood. The bubble transit times ranged from 60 to 365 (231.4±64.5) seconds. In all five rabbits, >80% of the bubbles had cleared from view <120 seconds after embolism. Regression analyses of the plateau decrement in left CBF and in the voltage amplitude of P2 (AP2) against transit time showed no significant relation between them.

Left CBF measured 2 minutes after the infusion was not significantly different from the preinfusion mean in the 25 μl embolism group (107.8±5.7%), the 400 μl embolism group (100.2±14.9%), or the control group (104.8±13.2%), and no significant differences existed among the groups at that time. Thereafter, left CBF decreased progressively in both
embolism groups (25 µl; \(F=2.6, \text{df}=69, p=0.02\); 400 µl; \(F=2.85, \text{df}=69, p=0.01\)) compared with the control group; this difference became significant after 45 (25 µl) to 60 (400 µl) minutes (Figure 3). The differences in left CBF between the embolism groups were never significant. At no time did left CBF in the control group differ significantly from the preinfusion mean. There were no significant differences in right CBF among the embolism and control groups or between the preinfusion and postinfusion means (data not shown).

The control group showed no significant variation in pial arteriole diameter during the experiments, whereas the 25 µl and 400 µl embolism groups showed significant \((F=2.03, \text{df}=69, p=0.04); F=5.8, \text{df}=69, p=0.005, \text{respectively})\) increases in external diameter \((24\pm3.2%, p<0.05; 41.9\pm10.8%, p<0.05, \text{respectively})\) 2 minutes after the infusion. The initial percentage dilatation in the two groups did not differ significantly. Pial arteriole diameters then slowly returned to the preinfusion means so that by 90 (25 µl) and 30 (400 µl) minutes diameters of the embolism groups did not differ from that of the control group (Figure 4).

As bubbles appeared in the pial vessels, the cortical somatosensory evoked response was suppressed. In the 25 µl embolism group this suppression was brief, and as the bubbles passed out of the exposed vessels (all were cleared <30 seconds after infusions) the cortical somatosensory evoked response returned to normal (Figure 1B). \(\text{AP}_2\) measured 2 minutes after the infusion of 25 µl air \((91.5\pm5.5%)\) did not differ significantly from either the preinfusion mean in these rabbits or \(\text{AP}_2\) in the control group at that time \((103.4\pm14.4%)\). Thereafter, \(\text{AP}_2\) progressively decreased in the 25 µl embolism group \((F=2.49, \text{df}=69, p=0.02)\) compared with the control group; this difference became significant after 75 minutes (Figure 5). \(\text{AP}_2\) in the control group never differed from the preinfusion mean, and the latency of \(\text{P}_2\) did not vary significantly at any time in any group (data not shown). Regression analyses of left CBF against \(\text{AP}_2\) in the 25 µl embolism group demonstrated a linear relation \((r=0.67, \text{df}=69, F=8.9, p=0.012)\) over the data range.

In contrast, in the 400 µl embolism group suppression of the cortical somatosensory evoked response persisted even after the bubbles passed out of view (Figure 1C). Although almost all bubbles had cleared by 2 minutes and left CBF at that time did not differ from the preinfusion mean and that in the control group (Figure 3), \(\text{AP}_2\) in these rabbits was still significantly \((F=2.85, p=0.01)\) reduced \((29.4\pm12.7%,es<0.05)\). \(\text{AP}_2\) had recovered slightly when it was measured again 15 minutes after the infusion \((49.6\pm8.1%)\). Thereafter there were no further changes in \(\text{AP}_2\) in this group (Figure 5). At all times after embolism \(\text{AP}_2\) in this group was significantly lower than the preinfusion mean and that in the control group; \(\text{AP}_2\) was also significantly lower than that in the 25 µl embolism group during the first 60 minutes after embolism. Regression analyses of left CBF against \(\text{AP}_2\) in the 400 µl embolism group showed no relation between the two.
Discussion

We describe the effect of different volumes of intracarotid air on pial arteriole diameter, cortical somatosensory evoked response, and CBF in rabbits.

The cortical somatosensory evoked response produced by stimulation of a rabbit's right forepaw is projected to a small area on the surface of the left cerebral hemisphere. The initial slope index of a hydrogen clearance curve measures the fastest blood flow in a small (approximately 8 mm³) region of the brain. Hence, we were able to observe the changes in CBF and cortical somatosensory evoked response in the very localized region in which we observed bubble passage. The decreases in both parameters seen in this region contrasted with data from the contralateral hemisphere of rabbits in the two embolism groups and both hemispheres of rabbits in the control group. These areas were unaffected, and therefore it appears that the bubbles caused these changes.

With none of the intracarotid doses of air we have tested up to 300 μl (10–125 μl/kg) are bubbles trapped in the exposed pial vessels; bubble transit is rapid (<5 seconds) and blood flow is restored immediately. At the other extreme, when air infusion is continued until bubbles permanently block the pial vessels, rabbits survive for only approximately 20 minutes.

A single intracarotid dose of 400 μl (160–190 μl/kg) air was needed to produce the intermediate stage of temporary bubble trapping, which was associated with inhibition of both neural function and CBF ipsilateral to the infusion; contralateral CBF was unaffected. This ipsilateral bubble effect has been previously described by ourselves and others. Within 2 minutes after embolism, however, 80% of all bubbles had cleared from view and CBF was 100.2% of the preinfusion mean, not different from that in the 25 μl embolism group (107.8%) or the control group (104.8%). The progressive decline in CBF to approximately half of the preinfusion mean in the 400 μl embolism group during the subsequent 45 minutes did not differ from that seen in the 25 μl embolism group.

The external diameters of the embolized vessels increased significantly, but again, changes in the 25 and 400 μl embolism groups did not differ significantly. Indeed, the dilatation seen in both groups did not differ significantly from that following a lethal dose of air, and the more prolonged dilatation was seen in the 25 μl embolism group. The effect of air embolism on both CBF and pial arteriolar diameter appears therefore to be independent of dose.

We have argued previously that the vasodilatation and the subsequent reduction in CBF cannot be explained by transient ischemia secondary to cerebral vessel occlusion, brain stem embolism, or excessive hydrostatic pressures within the vessels. Also, the decrease in CBF cannot be explained by changes in MABP, PAO₂, or Pao₂, all of which remained stable in our rabbits, or by changes in intracranial pressure, which does not increase in this open-brain model. We have also previously debated the possible mechanisms that may underlie the vasodilatation and CBF decrements. These mechanisms are not identified any more clearly by our studies, for two reasons. First, we can describe the behavior of only the bubbles seen in the exposed pial vessels, and second, the initial slope index method estimates only the fastest blood flow within the tissue sampled. Consequently, while small areas of reduced blood flow will not be identified, any demonstrated decrease in CBF using this method can be assumed to be significant.

Air embolism also suppressed the cortical somatosensory evoked response, indicating that sensorimotor cortex function was impaired, but this effect was dose dependent. In the 25 μl embolism group, the suppression was brief and limited to the period of bubble passage. The subsequent decline in voltages in this group correlated well with falling CBF (r=0.67). A coupling of brain function and CBF has also been demonstrated in cats after air embolism, and in dogs the recovery of cortical somatosensory evoked response after air embolism correlates well with CBF in the sensorimotor cortex. In contrast, 2 minutes after the infusion of 400 μl air, although CBF was 100.2% of the preinfusion mean and most bubbles had cleared, the cortical somatosensory evoked response did not recover and AP remained at <30% of the preinfusion mean. This deterioration was followed by an improvement during the next 13 minutes to 50% of the preinfusion mean, but no further improvement was seen. This profound postembolic inhibition of the cortical somatosensory evoked response was significantly greater than that after a 25 μl infusion, and neural function and CBF were clearly uncoupled. We did not identify the reasons for either this uncoupling of brain function and CBF or the sustained suppression of brain function. While these measures of CBF underestimate areas of low blood flow within the sampled tissue, it is noteworthy that the evoked response voltages and the initial slope index of CBF correlated after the infusion of 25 μl air.

The results from these three representative air doses (25 μl, 400 μl, continuous) are consistent with the natural history of air embolism of the brain in divers. Approximately 5% of such divers suffer cardiorespiratory arrest and die. This is analogous to the lethal dose of continuous air. In approximately 35% of the divers there is a sustained interruption of neural function, analogous to the dose of 400 μl air (temporary bubble trapping but sustained inhibition of cortical somatosensory evoked response). Finally, in the remaining 60% of such divers there is spontaneous recovery, often complete; this is analogous to the dose of 25 μl air (rapid bubble transit and recovery of cortical somatosensory evoked response). The subsequent decline in CBF and neural function following this 25 μl insult can also explain why some divers who recover relapse.
Indeed, the observed time frame in these rabbits is comparable with the peak occurrence of relapses in those patients with air embolism who initially respond to recompression therapy. This model of air embolism of the brain, therefore, appears to be suitable for further study, including the testing of potential therapeutic regimens.

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References


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