Arterial Air Embolism: Structural Effects on the Gerbil Brain

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SUMMARY Air injection into the carotid artery of adult mongolian gerbils caused, within 10 minutes, multifocal brain lesions. The extracellular spaces were widened and neurons, oligodendrocytes and myelin sheaths remained unchanged. The "delayed" effects of air embolism (first seen after 3 h) were similar to those observed in gerbils after unilateral carotid ligation. The histologic alterations after 3 h consisted of astrocytic swelling and shrinkage/necrosis of neuronal soma. The observations reported here illustrate the temporal and spatial separations that exist between a) brain water retention, and b) intraparenchymal entry of horseradish peroxidase. Both alterations can be a consequence of either decreased blood flow or arterial air embolism. Edema and protein leakage in each situation may be initiated by different mechanisms.

CEREBRAL AIR EMBOLISM, both arterial and venous, acquires considerable significance because of the increase in either diagnostic or reparative cardiovascular procedures that require atmospheric exposure of the intravascular surfaces. Forty of 340 patients after open-heart surgery had signs of arterial embolism, and 34 of these had either psychologic or neurologic deficits attributable to brain air embolism. 7 In a separate study, the incidence of air embolism to the brain following open-heart surgery was estimated at 6 percent. 2 Recent, comprehensive reviews of the English medical literature report the air embolism, and 34 of these had either psychologic or neurologic deficits attributable to brain air embolism. 1 In a separate study, the incidence of air embolism to the brain following open-heart surgery was estimated at 6 percent. 2 Recent, comprehensive reviews of the English medical literature report the rate of complications of percutaneous subclavian catheterization ranging from 0.4 to 9.9 percent. 8 Several reports of air embolism as an important complication of venous catheterizations have been published. 4 Venous air embolism to the brain, during posterior fossa craniotomy, occurs at a rate that ranges from 2.6 to 40 percent. 6

Air embolism is also a significant contributor to the number of deaths or illnesses resulting from rapid decompression. Seizures, focal neurologic deficits, un consciousness and other symptoms in healthy divers have been attributed to air embolism into either the carotid-vertebral or coronary arteries. 5 6 These deaths and symptoms could be the consequence of either cardiac or cerebral ischemia which, presumably, is induced by the transient mechanical occlusion of blood vessels by the air bubbles. 7

The present study was undertaken to a) define some of the ultrastructural brain changes induced by carotid air embolism, and b) compare in the same species, the changes secondary to air embolism with those resulting from ischemia induced by carotid-artery ligation.

Materials and Methods

The observations reported were made in 26 adult (50 to 78 gm body weight) Mongolian gerbils (Meriones unguiculatus) of either sex. Animals whose carotid artery was ligated and normal controls were anesthetized with ketamine (2.2 mg per animal) at the time of the neck surgery, and with sodium pentobarbital (3.5 mg per gerbil) at the time of the vascular perfusion. Each gerbil subjected to air embolization was anesthetized with 3.5 mg of sodium pentobarbital only.

Unilateral Carotid Artery Ligation. Eight gerbils had left carotid artery ligation 4 and were killed 30 min, 1, 2, 3, or 4 h later. Although a total of 13 gerbils had carotid occlusion, only those showing symptoms of unilateral ischemia are included in this study. There were 2 symptomatic gerbils in each group; those killed after 30 min could not be evaluated for lateralizing symptoms. Two gerbils served as sham-operated controls; they were subjected to all of the same manipulations except for the arterial clipping.

Air Embolism. Each of the 12 animals in this group received one injection of 0.05 ml of air into the right internal carotid artery; 9 the time when each animal was killed varied; from 10, 30, 60 min to 3 or 24 h after air injection. Two gerbils were killed at each of the above intervals, except for the 3 h period which included 4 gerbils. Four additional gerbils served as sham-operated controls; they had carotid puncture without air injection.

Each animal in all the groups described above received 50–85 mg of horseradish peroxidase (HRP, Sigma type II) in 0.5 ml Ringer's lactate. HRP was injected intravenously, either 5 min (20 animals) or 3 h (6 animals) before death.

All gerbils were killed under general anesthesia. The ascending aorta was perfused first with a solution of Ringer's lactate containing heparin (10 units/ml) and 0.1% procaine hydrochloride (380 mOsM), for an average period of 75 sec. Next, 100 ml of a mixture of 2.0% formaldehyde and 1.0% glutaraldehyde in a 0.15 M sodium phosphate buffer (1000 mOsM) was infused for an average period of 14 min. Perfusates were ad-
justed to a pH of 7.4, maintained at 25°C, and administered at a pressure of 130 cm of water.

Samples for microscopic examination (histology and electronmicroscopy) included 6 areas from 3 corresponding symmetrical sites in both hemispheres which included basal ganglia, hippocampus and insular neocortex.

Tissue samples measuring approximately 0.5 × 2.0 × 2.0 mm were rinsed at 4°C in 0.1 M sodium cacodylate, 0.2 M sucrose, pH 7.4. Histochemical demonstration of HRP was completed after incubating these tissue slices in 3,3′ diamino-benzidine-tetrahydrochloride (DAB) 50 mg in 100 ml of 0.05 M-Tris buffer containing 0.01% H2O2, pH 7.5 for 1 to 2 hours at 25°C.10 Tissue samples were rinsed in cacodylate-sucrose buffer, en bloc stained with uranyl acetate, dehydrated in ethanol, and embedded in epoxy resins. One micron thick sections were prepared for light microscopy. From these, appropriate areas for ultra-thin sectioning were selected and, after staining with lead citrate, they were examined in a Jeolco 100B electron microscope operating at 60 keV. The observations reported are based on the analysis of over 900 electronmicrographs.

**Results**

**Clinical Observations**

All 6 sham-operated animals displayed normal motor activity and normal behavior on awakening from anesthesia. In the air injected animals signs and symptoms secondary to unilateral carotid artery occlusion included ipsilateral circling (all 6 gerbils that survived one h or longer), weakness of contralateral front paw (5 gerbils), and intermittent, generalized, spontaneous tonic-clonic seizures (3 animals).

Of the 12 gerbils that had intracarotid air injections, 4 were sacrificed before they recovered from pentobarbital anesthesia. In the remainder, stupor (lasting a few hours) was noted in 4, persistent ipsilateral circling was evident in 6, and intermittent, generalized, myoclonic seizures in 4. At the time of sacrifice, 2 animals surviving 24 h, appeared neurologically normal except for persistent circling, which was mostly ipsilateral.

**Morphologic Examination of Brain**

Naked eye examination of formalin-fixed, coronally sectioned brains from gerbils with unilateral carotid-artery occlusion showed hemispheric asymmetry with unilateral swelling and pallor, particularly noticeable in the area of the basal ganglia. These changes were ipsilateral to the carotid occlusion and were more apparent in the 12-hour survivor than in the other 6.

Brains injected with air through the carotid artery showed, on coronal sections, moderate ipsilateral swelling with slight narrowing of the lateral ventricle. This was more apparent in the 24 h survivors whose brains had slight contralateral shift.

**Histologic and Ultrastructural Evaluations**

Samples of brain tissues from the sham-operated gerbils showed structural features in basal ganglia, hippocampus and neocortex (figs. 1, 2), which in every respect appeared identical to those of normal laboratory animals.11

Intracarotid air injection examined in animals killed after 10 to 30 min, led to the formation of multiple foci (average diameter 1.0 mm) of vacuolation which were randomly scattered throughout gray and white matter of both hemispheres (figs. 3, 4, 5). These foci were, however, more numerous in the hemisphere ipsilateral to the air injection. Portions of white matter in the internal capsule showed markedly enlarged extra-cellular space where myelin lamellae and axis cylinders remained unchanged (fig. 6). Oligodendroglia displayed normal structure in all samples examined. In addition to the enlargement of the extra-cellular space, there were occasional non-membrane bound circular spaces that suggested air bubbles (fig. 7). Widening of the extracellular space did not seem to change with increasing time. Occasional astroglial processes were swollen during the acute period (10 to 30 min). Scattered neurons in the neocortex and hippocampus appeared modestly condensed or "dark" (fig. 7).

In gerbils killed 3 hours after the air injection, scattered neocortical and basal ganglia neurons that were topographically unrelated to foci of vacuolation showed intra-cytoplasmic clearing and HRP accumulation in the cytosol. The majority of the brain lesions observed after 3 h were remarkably similar to those seen in brain ischemia, and consisted of massive swelling of perivascular astrocytic processes with pronounced shrinkage of neuronal perikarya (figs. 8, 9).

**Horseradish Peroxidase Extravasation**

After short intervals (10 to 30 min) after air embolism, HRP was seen in the endothelial cells of both capillaries and arterioles. In these cells, HRP could be seen in structures that correspond to either channels or vesicles. The nature of the endothelial pores or channels observed in both experimental groups was illustrated in a separate communication.19 Occasionally, HRP stained diffusely the endothelial cytosol (fig. 10). HRP leakage extended across the basal laminae into the extracellular space (fig. 10), where it was especially abundant in samples from animals that survived 3–24 h. The number of neurons containing HRP (figs. 11, 12) was greater in the 24 h survivors than in the gerbils killed after 10 min.

Tissue samples from the contralateral cerebral hemisphere from gerbils with unilateral carotid artery ligation showed features indistinguishable from those observed in the sham-operated controls. The tissues from the hemisphere ipsilateral to the arterial ligation displayed pronounced alterations that were multifocal but diffuse. These were more evident in the basal ganglia, although to a lesser extent were also visible in...
FIGURE 1. Pyramidal cell layer of hippocampus from a gerbil with a sham operation 30 min before death (400 X).

FIGURE 2. Neocortex (insula) from same animal as in figure 1 showing a capillary at the top and a neuronal perikaryon at the bottom (4,000 X).

FIGURE 3. Pyramidal cell layer of hippocampus, from a gerbil following air injection into the carotid 10 min before death. The upper portion shows focal injury characterized by massive enlargement of extra-cellular space and slight darkening of neuronal soma (400 X).

FIGURE 4. Neocortex, from a gerbil injected with air 10 min before death. A capillary is seen at right upper corner. The extracellular space is widened and only a few terminal boutons contact this neuronal perikaryon (9,000 X).
FIGURE 5. Same animal as in figure 4. Detail of neuronal soma making contact with 3 terminal boutons. Note very widened extracellular space (30,000 ×).

FIGURE 6. Internal capsule from a gerbil injected with air 30 min before death. There is massive enlargement of extracellular space, without myelin splitting or intrinsic axonal changes. Note absence of HRP (15,000 ×).

FIGURE 7. Neocortex of a gerbil, air embolism 30 min before death. The central circular clear space is suggestive of an air bubble. There is also widening of the extracellular space and slight increase in density of some neuronal soma (450 ×).

FIGURE 8. Basal ganglia of a gerbil, air injection 3 h before death. There is marked enlargement of a pericapillary astrocytic processes without visible changes in the glial mitochondria. Extracellular space is not widened and there is no extravasation of HRP (9,000 ×).
FIGURE 9. Neocortex from a gerbil injected with air 24 h before death to demonstrate enlargement of astrocyte (arrowhead), prominent vacuolation of neuropil and several dark neurons (450 ×).

FIGURE 10. Cerebral neocortex from a gerbil injected with air 10 min before death. HRP can be seen in endothelial cell, extracellular space, axonal neurotubules and astrocytic processes. All of these are free of swelling (5,000 ×).

FIGURE 11. Neocortex of a gerbil, air and HRP injected 3 h before death. Abundant deposits of HRP are visible in extracellular spaces and neuronal soma (Dark Field 1,000 ×).

FIGURE 12. (Same animal as in figure 11) demonstrates abundant HRP in nucleoplasm and cytosol of a cortical neuron that also shows electron-lucent areas beneath plasma membrane (12,000 ×).
the hippocampus of the gerbils killed after 3 and 4 h, respectively. The changes most consistently observed in the areas of sponginess included vacuolation of the neuropil, swelling of astrocytic nucleus, increased lucency of astrocytic cytoplasm, shrinkage/condensation of neuronal perikaryon (figs. 13, 14), and relative preservation of oligodendrocytes and myelin sheaths. Widening of the extracellular space in either gray or white matter was not seen in the samples examined.

Discussion

Unilateral carotid-artery ligation in gerbils caused neurologic abnormalities and brain structural changes previously reported by others as characteristic of unilateral brain ischemia in the carotid artery territory. As in other models of acute occlusive ischemia, there was abundant swelling of astroglia accompanied by shrinkage and other structural changes in neurons. A limited number of abnormalities were evaluated in this study of arterial air embolism. They included ultrastructural alterations in neuronal perikaryon, axons, myelin sheaths, endothelial cells, astrocytes and extracellular space.

Two chronologically separate effects were observed: 10 minutes after the arterial injection of air there was multifocal enlargement of the extracellular space while neuronal perikarya appeared minimally altered. At later intervals (3 h to 24 hr), swelling (i.e.: increased electron lucency) affecting primarily perivascular astrocytic processes was accompanied by neuronal shrinkage and hyperchromasia. All of these changes are similar to acute neuronal injury secondary to ischemia. The injection of air into the carotid artery of rats results in bi-phasic alterations of brain energy metabolites; the initial set of abnormalities occurs at the time of injection, while a completely different set becomes evident during the recovery phase. Interestingly, hyperbaric oxygen can improve the energy state of brain tissue embolized with air and eliminates the mortality, which can reach 53% in the untreated rats. Treatment with hyperbaric oxygen has been successfully applied in several instances of human arterial air embolism. Circulating gaseous bubbles may induce endothelial damage, which is not caused either by hypoxia or ischemia. Such direct endothelial injury can lead to intravascular coagulation and occlusion. Dogs, after being embolized with air, and prophylactically treated to remove circulating plasma cryoprecipitates, had less brain damage than dogs in the untreated group.

Functional endothelial injury is shown by transient, increased permeability to macromolecules such as

Figure 13. Basal ganglia ipsilateral to side of carotid-artery occlusion (3 h duration) showing slight condensation of neuronal soma with marked enlargement and increased lucency of astrocytic processes (9,500 X).
Figure 14. Representative sample of a basal-ganglia neuron from gerbil with carotid artery ligated 4 h before death. Increased darkening of neuronal perikaryon and discontinuities in plasma membrane are apparent (9,500 ×).

Abnormalities in the transport of low molecular-weight substances e.g. glucose and sodium chloride are present in more extensive areas of the brain and persist longer than those involving macro-molecular transport. In addition, air embolism causes increased brain water uptake and increased brain glucose utilization, which are unrelated to changes in cerebral blood flow. The results of experiments comparing leakage of macromolecules in normotensive and hypertensive rats embolized with air suggest that protein extravasation after air embolism is caused by factors other than hemodynamic alterations. Moreover, alterations in blood flow, secondary to the injection of air into both carotid arteries, lasted only one to 3 min after the injection of the air bolus.

Circulating proteins may enter the brain tissue through trans-endothelial pores or channels. Increased protein permeability has been demonstrated in capillary endothelium of gerbils that were either injected with air or had one carotid artery ligated. Except for increased numbers of either transendothelial pores or pinocytotic vesicles, we saw no structural alterations in the endothelial cells of gerbils after injection with air.

There was no spatial concurrence between the sites of extravasation of protein and the foci of enlargement of the extracellular space (figs. 4, 6, 10). Temporal discrepancy between these phenomena has been observed in experiments with middle cerebral artery occlusion in cats. In these animals, the ipsilateral hemisphere showed a maximal increase in volume 3 to 4 days after the arterial occlusion, and the escape of protein-bound tracers peaked several days later. Analysis of sequential brain scintigrams in monkeys after middle cerebral artery occlusion showed that the maximum radionuclide uptake (i.e.: macromolecule leakage) occurred approximately 20 days after the arterial occlusion or at a time when all excess water would have been expected to have disappeared.

Experimental cerebral air embolism is said to induce no detectable structural changes in the brain, cause necrosis and demyelination, induce extensive red softening, or lead to multifocal infarctions. Our observations suggest that carotid arterial air embolism has a 2 step effect on the gerbil brain. Shortly after the air injection (i.e.: 10 min) there is multifocal enlargement of the extracellular space that seemingly reflects abnormalities in endothelial function. Approximately 3 hours after air embolism the parenchymal changes are similar to those observed in gerbils with carotid artery occlusion.

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References

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