Brain Edema and Blood-Brain Barrier Permeability Following Quantitative Cerebral Microembolism

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SUMMARY Cerebral microemboli were formed in rats by injecting 4,000 carbonized microspheres, 50 ± 10 μ in diameter, labelled with 85Sr, into the internal carotid artery. The use of radioactive microspheres as embolic agents enabled the number of microspheres to be determined in each cerebral hemisphere. The microspheres were mainly distributed in the cerebral hemisphere on the side of the injection. In 61 rats this hemisphere contained 582 ± 20 microspheres against 99 ± 9 in the contralateral hemisphere.

Brain edema was assessed by measuring brain content of water, sodium and potassium. Blood-brain barrier (BBB) permeability was determined by brain accumulation of 131I-albumin. In the ipsilateral hemisphere brain edema and an increase in BBB permeability appeared 6 hours after embolization and progressed up to 48 hours. Twenty-four hours after embolization, significant correlations were observed between the microsphere content of the cerebral hemispheres and 1) the increase in water and sodium levels, 2) the decrease in potassium level, 3) the increase in BBB permeability.

The study of these correlations should make it possible to ignore the poor reproducibility of embolizations and to analyze with increased accuracy the results of various experiments.

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Methods

Production and Evaluation of Embolization

Male Iffa Credo rats weighing 240–260 g were anesthetized with intraperitoneal chloral hydrate (360 mg/kg). After exposure of the left common carotid artery, dissection was carried above the bifurcation and the external carotid artery was ligated. Carbonized microspheres (3M, 50 ± 10 μ diameter, labelled with 85Sr) were suspended in 20% dextran and injected into the internal carotid artery. The spheres were injected by hand, within 30 seconds, into the artery without interruption of the blood flow and flushed with 0.1 ml 20% dextran. Thirty seconds after the end of the injection, the artery was ligated above and below the puncture site, the needle withdrawn and the wound closed. In pilot experiments, carotid pressure was determined. The effects of embolization were evaluated by assessment of brain edema and blood-brain barrier (BBB) permeability and correlated with microsphere content.

AMONG THE WIDE variety of experimental models for brain ischemia, embolic methods have been used which inject microemboli into the carotid arterial system to occlude blood vessels. The effects of different sized microemboli on the vascular system and parenchyma of the brain have been studied using histological techniques in dogs, rabbits and cats. In the rat, the metabolic effects of brain embolization have been studied after intracarotid injection of calibrated 80 μ or 35 μ microspheres. The results of these studies showed alterations in brain water, energy metabolites and monoamine levels in the cerebral hemisphere on the same side as the microsphere injection but also in the contralateral hemisphere.

Such experimental models are useful to study the effects of agents or therapy influencing cerebral infarction and ischemic swelling. For such an experimental model to be most useful it is important that the extent and localization of embolization be highly reproducible, a feature that has not been thoroughly studied and reported on in the past. In previous work, we evaluated the distribution of radioactive 15 μ microspheres injected into the rat internal carotid artery. Results showed that about 70% of the injected microspheres were located in extracerebral tissues and that the intracerebral microspheres were largely distributed in the cerebral structures on the side of injection, but variable amounts of microspheres were found in the contralateral cerebral structures. This variability emphasizes the unpredictability of collateral flow through the circle of Willis. Consequently, it is difficult to be certain whether the alterations which have been reported in the contralateral cerebral hemisphere of embolized rats are due to the phenomenon of diaschisis or to the emboli.

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In the present investigation, emboli have been created using radioactive microspheres, enabling the number of microspheres in each hemisphere to be determined. The effects of embolization were evaluated by assessment of brain edema and blood-brain barrier (BBB) permeability and correlated with microsphere content.
bolization of from 5 min to 48 hours. The brain was removed, the cerebellum and the brain stem were discarded, the right and the left hemisphere were placed into tared vials and their radioactivity was determined in a scintillation crystal well counter (Nuclear Chicago). In order to calculate the number of microspheres contained in each sample, the mean radioactivity in one microsphere (about 3-5 cpm) was determined by simultaneous counting of the microsphere suspension in a hemocytometer and in the scintillation counter.

Assessment Brain Edema and BBB Permeability

Brain edema was evaluated by the content of water, sodium and potassium in the cerebral hemispheres. Water content (g H₂O/100 g of fresh weight) was determined by difference after drying at 95°C to a constant weight. Sodium and potassium determinations (mEq/kg of fresh weight) were made in the dry hemispheres using flame photometer.

Blood-brain barrier permeability was studied by estimation of the transfer of ¹²⁵I-albumin from blood into brain. Rats received i.v. injection of about 5 µCi of ¹²⁵I-albumin (Centre National de Transfusion Sanguine, Paris) immediately after embolization and were decapitated at various times after injection of the tracer. The ¹²⁵I radioactivity in plasma and cerebral hemispheres was measured and the uptake of ¹²⁵I-albumin was calculated by the ratio:

\[
\frac{\text{¹²⁵I cpm/g cerebral tissue}}{\text{¹²⁵I cpm/g plasma}} \times 100
\]

For simultaneous counting of ⁸⁵Sr and ¹²⁵I, the energy windows utilized were 400-600 keV and 20-50 keV respectively. The activity of each isotope was calculated according to the method of Rudolph and Heymann.

The relationships between the number of microspheres in the cerebral hemispheres and the levels of water, sodium and potassium or the ¹²⁵I ratio were studied by linear regression analysis. The Student's t-test was used to analyze statistical significance of differences between group means. A p value of less than 0.05 was considered significant.

Results

Embolized animals were lethargic, showing asymmetrical posture and ipsilateral ptosis and occasionally exhibiting circling movements and rolling seizures. Ten to 20 percent mortality occurred within 48 hours.

Distribution and Reproducibility of Embolization

In 61 rats submitted to injection of 4,000 microspheres into the left internal carotid artery and sacrificed 24 hours later, 582 ± 20 microspheres were found in the left hemisphere and 99 ± 9 in the right hemisphere. Six animals showed abnormal distribution of microspheres between the 2 hemispheres, the right hemisphere containing more than 200 microspheres.

Effect of Embolization on Brain Water Content

Figure 1 shows the changes of water content in the cerebral hemispheres at 5 and 15 min, 2, 6, 12, 24 and 48 hours after embolization. Water content in the cerebral hemispheres of non-embolized rats was found equal to 78.88 ± 0.10% (n = 8). In the left hemisphere, water level began to increase significantly 6 hours after embolization (79.89 ± 0.17%, n = 6). It reached 81.38 ± 0.28% (n = 6) at 12 hours, 81.96 ± 0.25% (n = 8) at 24 hours and 82.85 ± 0.41% (n = 8) at 48 hours. In the right hemisphere, water content was significantly increased, as compared to control values, only at 24 hours (79.41 ± 0.06%, n = 8) and 48 hours (79.62 ± 0.13%, n = 8).

It is known that strong irradiation of cerebral tissue leads to the development of brain edema and in order to verify that brain edema, which was observed after embolization, was not due to the presence of radioactive microspheres, we measured the water level in cerebral hemispheres 24 hours after an embolization performed with non-radioactive 50 µ microspheres. Under these conditions, water content (n = 8) was found equal to 82.04 ± 0.33% in the left hemisphere and 79.41 ± 0.18% in the right hemisphere. These values are not significantly different from those which were obtained using ⁸⁵Sr-labelled microspheres.

Effect of Embolization on BBB Permeability

The ¹²⁵I accumulation in brain at various times after injection of ¹²⁵I-albumin is shown in figure 2. In the cerebral hemispheres of control rats, the ¹²⁵I brain/blood ratio remained relatively constant during 48 hours and varied between 1.5 and 2. In the left hemisphere of embolized rats, this ratio was significantly different from control values as early as 6 hours and it increased linearly with time up to 48

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Changes with time of water content in the left and right cerebral hemispheres of embolized rats (injection of 4,000 microspheres into the internal carotid artery). Each point is the mean ± SEM of 6 to 8 determinations.

*Significant difference between right and left hemisphere.
Correlation between Intensity of Brain Edema and Degree of Embolization

The intensity of brain edema was estimated through the water, sodium and potassium levels and the degree of embolization through the number of microspheres in the cerebral hemispheres. Twenty-four hours after embolization, significant correlations were observed between the degree of embolization and the water level \( (r = 0.95, p < 0.001, \text{fig. 3}) \) and the sodium level \( (r = 0.93, p < 0.001, \text{fig. 4}) \). A significant inverse relation was observed between the degree of embolization and the potassium level \( (r = -0.94, p < 0.001, \text{fig. 5}) \).

Correlation between BBB Permeability and Degree of Embolization

Twenty-four hours after embolization, a highly significant correlation was observed \( (r = 0.96, p < 0.001, \text{fig. 6}) \) between the degree of embolization and the increase in BBB permeability assessed by the \(^{125}\text{I}-\text{albumin brain/blood ratio.}\)

Discussion

The intracarotid injection of calibrated microspheres is a relatively simple method of producing brain injury in the rat. This method has been used in previous studies\(^6\), \(^7\) which differed from one another in the microsphere diameter, the amount of injected microspheres and the injection technique. Siegel et al.\(^8\) injected 5,000 microspheres, 80 ± 20 μ in diameter, into the common carotid artery via carotid puncture and reported that this procedure resulted in 10 to 20 percent mortality within 48 hours. The model described by Kogure et al.\(^7\) used the injection of 35 ± 5 μ microspheres into the catheterized internal carotid artery. The authors did not specify the number of injected microspheres but only their weight (0.38 mg). Such an injection caused about 60 percent mortality within 24 hours. Under our experimental conditions, 4,000 microspheres, 50 ± 10 μ in diameter, were injected into the internal carotid artery and led to 10 to 20 percent mortality within 48 hours.

The use of radioactive microspheres allows the determination of the intensity of the embolization and its distribution between the 2 cerebral hemispheres. The present results show that the hemisphere located on the side of the injection contained about 6 times more microspheres than the contralateral hemisphere. However, about 10 percent of the animals had abnormal distribution of microspheres. The cerebral localization of the microspheres was not systematically evaluated by Siegel et al. and Kogure et al. who used

\[ y = 76.828 + 0.0051x \]

\[ y = 46.341 + 0.028x \]
unlabelled microspheres as embolic agents. These authors only reported the results of pilot studies performed with radioactive microspheres. Siegel et al. observed that microspheres were distributed to both hemispheres but found a large degree of variability. The ipsilateral hemisphere contained 33.4 ± 15.4 percent of the injected spheres per gram of tissue whereas the corresponding value in the contralateral hemisphere was 8.0 ± 11.2 percent (mean ± SD, n = 11). Kogure et al. reported that all the microspheres became lodged in the ipsilateral hemisphere. These differences are probably related to the injection conditions. In our experimental conditions, the assessment of the degree of embolization is absolutely necessary when comparisons must be made between different series of experiments.

As evidenced by the increase of brain water, cerebral edema developed following embolization. Our results show that in the ipsilateral hemisphere edema was apparent at 6 hours and progressively increased up to 48 hours. In the opposite hemisphere, the edema which occurred 24 hours after embolization was also observed by Kogure et al. and was interpreted as the consequence of a water migration from the other hemisphere. Under our experimental conditions, it was due to the slight embolization of this hemisphere, as evidenced by the correlation at 24 hours between the water content and the number of microspheres in the cerebral tissue. Kogure et al. reported also that water content markedly increased in the ipsilateral hemisphere 5 min after embolization and returned to a normal value after 15 min. This early and transient change in water content was not observed in the present study.

Alteration in BBB permeability following microembolization has been histologically studied using dye staining. By assessing the brain albumin space, Siegel et al. reported a dissociation in the time between the onset of edema and the BBB dysfunction. Within 4 hours of embolization, edema was present without associated evidence of increased permeability to albumin which was significant only 8 to 16 hours after embolization. These authors suggested that 2 different mechanisms produced cerebral edema with microembolization. In the first hours following embolization, edema was present without alteration in BBB permeability. Our results do not support this hypothesis, as the development of brain edema occurred with BBB dysfunction. Similar conclusions were obtained by Plum et al. using another experimental model of ischemic encephalopathy, induced by exposing rats with unilateral carotid ligation to low oxygen tension. They found trypan blue staining of brain tissue in all animals with edema, 5 and 24 hours after injury.

Among the experimental models of brain ischemia, microembolic methods have not been much used. This is perhaps due to the difficulty in obtaining reproducible embolizations. The present results show that these difficulties can be reduced by the use of radioactive microspheres as embolic agents. The correlations observed between the effects of embolization and its intensity allow more accurate assessment of the effect of therapy on brain edema.

References

Total Cerebral Ischemia: A New Model System for the Study of Post-Cardiac Arrest Brain Damage

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SUMMARY  The pathophysiology of post-cardiac arrest brain damage is not well understood. Many of the model systems presently used to study global ischemia have serious limitations. A new model system for total cerebral ischemia (TCI), using aortic and inferior vena caval occlusion balloons, is described. This model system produces verifiable TCI and avoids surgical invasion of the thorax or the use of vasoactive drugs. It does not impede cerebral venous return and protects the cardiopulmonary system from damage. This model system can be used to study the efficacy of various therapeutic interventions following a standardized CNS global ischemic insult.

CARDIAC ARREST is a frequent critical care emergency, which can arise from dysfunction of many different organ systems. Cessation of effective myocardial function is a "final common pathway" for many disparate processes. Attempts to resuscitate patients following cardiac arrest have been reported for centuries. In 1878 Boehm demonstrated that compression of the thorax in animals could cause circulation of blood. However, it has only been in the last 20 years that an effective clinical regime for cardiopulmonary resuscitation has been widely available. This required the introduction of external electrical defibrillation and a demonstration of the effectiveness of closed chest cardiac massage. More effective supportive therapy and sophisticated monitoring capabilities have also increased the possibility of survival of patients in such critical care situations.

Approximately 50% of patients experiencing cardiac arrest in a modern intensive care unit are initially successfully resuscitated. However, only a total of 20% of all patients experiencing cardiopulmonary arrest in this setting survive to discharge from the hospital. The ultimate prognosis for a patient following cardiac arrest is often difficult to assess in the immediate post-arrest period, as survival with full restoration of function may be seen despite early evidence of severe damage to the nervous system. However, many of the patients who die following initially successful resuscitation remain in deep coma with markedly abnormal neurological examinations and electroencephalograms.

The critical vulnerability of the central nervous system (CNS) to hypoxic/ischemic injury has been a cornerstone of classical medical doctrine. In the traditional view, the normothermic brain tolerates only 4–8 minutes of total cerebral ischemia before significant permanent damage is caused. Recently, however, Hossmann and colleagues have demonstrated recovery of significant neurologic function in animals following 30–60 minutes of "total cerebral ischemia." Cerebral microcirculatory changes may be a limiting factor in the tolerance of the CNS to ischemia. Ames and his colleagues first popularized the concept of the "no reflow phenomenon." They described a failure to reestablish flow when the perfusion pressure in the CNS was restored following circulatory arrest. This phenomenon is seen not only in the CNS but also in the myocardium. The pathophysiological and anatomical bases for these changes remain obscure. Safar and colleagues have reported recovery of significant neurologic function in dogs following 12 minutes of ventricular fibrillation if vigorous "anti-no-reflow" therapy is instituted.

Many experimental model systems have been employed to study total cerebral ischemia, including intrathoracic cross-clamping of the aorta, inflation of an external pneumatic cervical pressure cuff, inducing ventricular fibrillation, and clamping various arteries (carotid/vertebral) in the neck. Many of
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