Brain Edema and Cerebrovascular Permeability During Cerebral Ischemia in Rats

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Focal cerebral ischemia was produced by occluding the left middle cerebral artery in 769 rats. Permeability of the blood–brain barrier to small or large molecules was evaluated qualitatively using Evans blue or sodium fluorescein and quantitatively using the transfer indexes of iodine-125–labeled bovine serum albumin or [14C]sucrose. Water content was determined using wet and dry weights and sodium and potassium contents using flame photometry. Cortical tissue in the middle cerebral artery territory was sampled ≤14 days after occlusion. A significant increase in the albumin transfer index was first found 12 hours after occlusion, and the index remained approximately the same until water content peaked 3 days after occlusion. In contrast, the sucrose transfer index increased gradually, significantly correlated with increases in the water and sodium contents. Tissue staining by sodium fluorescein was more extensive than that by Evans blue. As edema fluid decreased gradually 4–10 days after occlusion, the albumin and sucrose transfer indexes increased markedly. These findings indicate that disruption of the blood–brain barrier to small molecules is accompanied by accumulation of edema fluid during the later stages of ischemia. Opening of the barrier to serum protein is probably related to the resolution of edema. (Stroke 1990;21:582–588)

Movement of water from a capillary into brain tissue probably follows the Starling equation. Many factors including cerebrovascular permeability, the hydraulic conductivity of capillaries, hydrostatic and osmotic pressure gradients, and tissue compliance and resistance, modify edema formation. However, the pathophysiology of ischemic brain edema remains poorly understood.

We recently demonstrated that a hydrostatic pressure gradient develops between blood and brain tissue soon after the onset of focal ischemia, associated with an early accumulation of edema fluid. As the gradient dissipates, an osmotic gradient develops between blood and the ischemic brain tissue, causing further accumulation of edema fluid. As the ischemic injury progresses, edema fluid accumulates in compliant brain parenchyma and then migrates through conductive tissue into the cerebrospinal fluid spaces.

Normal function of the blood–brain barrier (BBB) is altered by ischemia. Increased permeability of the BBB is associated with severe ischemic injury, occurring with some delay after the insult. Reperefusion of tissue after transient ischemia accelerates the development of abnormal vascular permeability and worsens postischemic edema. On the other hand, how altered vascular permeability affects the formation and resolution of edema in ischemic tissue not subjected to reperfusion continues to be unresolved.

Our present experiment was designed to study how changes in BBB function relate to the formation and resolution of edema fluid. We sought to clarify the relation between the contents of water and sodium in the brain and BBB permeability to small and large molecules in ischemic tissue after permanent arterial occlusion.

Materials and Methods

We anesthetized 769 adult male Sprague-Dawley rats weighing 300–400 g with 40 mg/kg i.m. ketamine hydrochloride and 10 mg/kg i.m. xylazine. Oxygen was given via a close-fitting face mask. Mean±SD body temperature was maintained at 37±0.5°C with an electric lamp. The middle cerebral artery (MCA) was approached by a modification of the method of Tamura et al. The rats were placed in the lateral position. The MCA was exposed by the transretroorbital approach, without damaging the zygomatic bone, retracting the temporalis muscle. After the
dura was opened, the MCA was occluded with micro-bipolar coagulation over approximately 2 mm proximal and distal to the olfactory tract. The dura opening was sealed with oxidized cellulose, the soft tissue was reapproximated, and the wound was closed. Sham operation was performed the same way, except the MCA was not occluded. The sham-operated and unoperated rats served as controls. The rats were allowed to recover from anesthesia and were returned to their cages, where they were permitted free access to food and water.

The rats were anesthetized a second time 60 minutes before decapitation. Cannulas were placed in the femoral artery and vein to measure systemic arterial blood pressure, to sample arterial blood gases and hematocrit, and to administer radioactive or dye tracers. Arterial blood pressure, arterial blood gases, hematocrit, and concentrations of sodium and potassium in the plasma were measured before injection of tracers or death.

The water content of brain tissue was measured by the wet and dry weight method in 149 rats. The rats were decapitated at 1, 3, 6, and 12 hours and 1, 2, 3, 4, 5, 6, 7, 8, 10, and 14 days after MCA occlusion. Samples of brain tissue were taken from cortex supplied by the MCA in both hemispheres, and were placed in preweighed crucibles and weighed. The samples were dried to a constant weight in an oven at 90–100°C for 2 days. Water content was calculated as [(wet−dry weight)+wet weight]×100%. The dehydrated samples were homogenized and digested with 0.75N nitric acid for 7 days. The concentrations of sodium and potassium ions in brain tissue were determined by flame photometry with lithium as the internal standard and expressed as milliequivalents per kg dry weight.

Permeability of the BBB to small and large molecules was assessed qualitatively using intravenous 2% Evans blue (mol wt 6,900, 20 mg/kg) in 178 rats and 10% sodium fluorescein (NaFl, mol wt 332, 2.0 ml/kg) in 167 rats, respectively. These tracers were injected 30 minutes before decapitation. Ten to 12 rats were killed 3, 6, 12, and 18 hours and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 14 days after MCA occlusion. Two rats were killed 6 and 12 hours and 3, 6, and 10 days after sham operation. The brains were cut in the coronal plane, and the surfaces of the slices were grossly inspected. A four-point grading scale was used to assess permeability of the BBB: 0, no staining; 1, small area with faint staining; 2, medium-sized area with moderate staining; and 3, extensive area with dark staining. The incidence of BBB permeability of ≥1 was calculated as a percentage of the rats killed at each interval after MCA occlusion.

BBB permeability to serum protein and small molecules was assessed quantitatively using radioactive tracers given 30 minutes before decapitation. Rats were killed 3, 6, 12, and 18 hours and 1, 2, 3, 4, 6, 8, 10, and 14 days after occlusion.

BBB permeability to serum protein was evaluated using 15 μCi of iodine-125-labeled bovine serum albumin (125I-BSA; New England Nuclear, Boston, Massachusetts) in 135 rats. Arterial samples were obtained to determine radioactivity in the blood before decapitation. The cerebral vasculature was perfused with 50 ml of 0.9% saline through the left ventricle at 130 cm H₂O; brain tissue samples were then taken from the cortex supplied by the MCA in both hemispheres. Radioactivity in the samples of arterial blood and brain tissue was determined with a gamma scintillation counter (Auto Gamma 5650, Packard Instrument Co., Inc., Meriden, Connecticut). The transfer index of 125I-BSA from blood into brain was calculated by dividing the radioactivity of brain tissue (as counts per minute per gram) by that of blood (as counts per minute per milliliter) and expressed as a percentage of that in contralateral tissue.11,12

BBB permeability to small molecules was evaluated using 15 μCi of carbon-14-labeled sucrose (14C-sucrose) in 140 rats. Arterial samples were obtained to determine radioactivity in the blood before sacrifice. After decapitation, brain tissue samples of both MCA territories were transferred into vials and dissolved with Protosol tissue solubilizer. Radioactivity in the samples was determined with a beta scintillation spectrometer (LS-9000, Beckman Instruments, Inc., Fullerton, California) and corrected for quenching with an internal 14C-sucrose standard. The concentration of tracer in the plasma exceeded that in brain tissue. The transfer index of 14C-sucrose from blood into brain was calculated by dividing the radioactivity of brain tissue (as disintegrations per minute per gram) by that of blood (as disintegrations per minute per milliliter) and expressed as a percentage of that in contralateral tissue.13

BBB status was correlated with water content of ischemic brain tissue using measurement of specific gravity in same rats with studies of radioactive tracers. Two small samples (1 mm³) dissected from cortical tissue after decapitation were dropped into a gradient column for specific gravity determination. Brain specific gravity water content was calculated and expressed as percent water. An increase in water content was determined by comparison with corresponding regions of the contralateral hemisphere and expressed as milligrams per gram of brain tissue.14 The remaining brain tissue was used for the measurement of radioactivity.

Statistical significance of the results was determined using Student's t test; p<0.05 was considered significant. All values are expressed as mean±SEM.

Results

Physiologic data for 10 unoperated controls and 229 rats decapitated at various times after MCA occlusion with studies of BBB permeability to radioactive tracers are summarized in Table 1. Compared with the unoperated controls, mean arterial blood pressure (MABP) did not change after MCA occlusion, remaining at approximately 100 mm Hg. arte-
Figure 1. Graph of mean ± SEM water contents in ipsilateral (●, n=8–10) and contralateral (○, n=8–10) hemispheres of rats killed 1 hour (h) to 14 days (D) after middle cerebral artery occlusion (MCAO) or sham operation (△, n=5 at each point). C, unoperated control rats (n=5). Water content increases progressively and reaches maximum 3 days after MCAO. *p<0.01 different from △ and ○ by Student’s t test.

Table 1. Physiologic Variables in Rats After Middle Cerebral Artery Occlusion

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<th>Time after occlusion</th>
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(n=8, p<0.01) 14 days after occlusion. Plasma potassium content did not change during the 14 days (4.29±0.04 meq/kg; n=119).

There was no visible staining of the brain by Evans blue or NaFl in 10 sham-operated controls. Extravasation of Evans blue was first seen in one of 11 rats killed 12 hours after occlusion, whereas NaFl was first evident in the brain of one of 10 rats killed 6 hours after occlusion. During the progressive increase in water content for 3 days after occlusion, the incidence of Evans blue and NaFl extravasation was 13.58% in 81 rats and 18.57% in 70 rats, respectively (Figure 3). The area stained by Evans blue was very small and the staining was very light, located mostly in the proximal portion of the core of the MCA territory. The extent and location of NaFl staining in the ischemic tissue was generally more extensive than that of Evans blue. After the period of maximum water content, the incidence of Evans blue staining was 32.61% in 92 rats killed 4-14 days after occlusion (Figure 3). The grade of Evans blue staining at this time was a little greater than that during the first 3 days after occlusion. In contrast, the incidence of NaFl extravasation was 79.35% in 92 rats killed 4-14 days after occlusion, greater than that of Evans blue or NaFl in rats killed ≤3 days after occlusion.

The transfer indexes of 125I-BSA in five unoperated controls, 20 sham-operated controls, and 110 rats killed ≤14 days after MCA occlusion are shown in Figure 4. There was no difference in 125I-BSA transfer index between unoperated and sham-operated controls. The transfer index of 125I-BSA from blood into brain did not increase significantly ≤6 hours after occlusion. Compared with unoperated or sham-operated controls, the index first increased 12 hours after occlusion. This small but significant increase was maintained at approximately the same level for up to 3 days after occlusion. The transfer index of 125I-BSA then increased markedly 4-10 days after MCA occlusion. The maximum 125I-BSA transfer index was 9.81±2.5×10⁻²% (n=9, p<0.01) 8 days after occlusion.

The transfer indexes of 14C-sucrose in five unoperated controls, 16 sham-operated controls, and 119 rats killed ≤14 days after MCA occlusion are shown in Figure 5. The transfer index of 14C-sucrose in unoperated controls was 1.09±0.06×10⁻²%, not different from that in sham-operated controls. A significant increase in the 14C-sucrose transfer index was first found 12 hours after occlusion. The 14C-sucrose transfer index then increased gradually, reaching a
maximum (4.95±0.94×10^3%; n=10, p<0.01) 8 days after occlusion. The transfer index then decreased to 3.33±0.72×10^2% 14 days after occlusion (n=9, p<0.05).

The 125I-BSA and ^14^C-sucrose transfer indexes were compared with water contents in 116 rats killed ≤3 days after MCA occlusion. There was no correlation between the 125I-BSA transfer index and water content (r=0.39, n=56). In contrast, the ^14^C-sucrose transfer index correlated with water content (r=0.63, n=60; p<0.05) until water content reached a maximum (Figure 6).

**Discussion**

We have demonstrated that the amount of edema fluid increases progressively in ischemic brain tissue within 1 day after MCA occlusion, reaches a plateau for the next 2 days, and then decreases gradually. These findings confirm those of previous studies showing that brain edema is maximal 1-3 days after the onset of ischemia, then resolves.12,15

Formation of ischemic brain edema depends primarily upon the duration and severity of the ischemia.16 Other factors also affect edema formation. We have recently demonstrated that the formation of brain edema contributes to a hydrostatic pressure gradient between blood and brain tissue soon after the onset of ischemia, followed by the development of an osmotic pressure gradient.3-5 These pressure gradients dissipate with time as ischemic injury progresses. Edema fluid continues to accumulate in the ischemic zone, even as the pressure gradients begin to resolve.

An increase in water content is accompanied by the passage of serum protein from blood into brain in the vasogenic edema associated with brain tumors or severe cortical contusion.17 Extravasation of serum proteins contributes to the retention of edema fluid in brain tissue. In cerebral ischemia, BBB breakdown to proteins alone probably aggravates the preexisting cytotoxic water increment.18 However, recent studies show a discrepancy between edema formation and changes in BBB permeability to proteins in animal models of cerebral ischemia.8,12

The BBB during ischemia has been evaluated histologically, employing large tracer molecules such as Evans blue-albumin or horseradish peroxidase.7 NaFl has also been used as a micromolecular dye in BBB studies. Wolman et al19 have shown that NaFl exists in an almost-free state when given intravenously, whereas Evans blue binds firmly to plasma proteins. Therefore, we measured BBB permeability using the visual dye tracers Evans blue and NaFl. Extravasation of these dyes provided qualitative information about vascular permeability to relatively small and large molecules.

More recently, BBB permeability has been determined quantitatively using [14C]aminoisobutyric acid autoradiography or the product of capillary permeability and surface area.20,21 These values must be calculated utilizing a specific equation, including a correction for the amount of intravascular tracer, which is determined differently. Under pathologic conditions, in particular, accuracy of the calculated blood-to-brain transfer depends on.
blood flow, capillary blood volume, back-diffusion of the tracer from brain into blood, and intraparenchymal tracer diffusion.

We evaluated the change in cerebrovascular permeability with the radioisotope tracers $^{125}$I-BSA and $^{14}$C-sucrose. Capillary permeability was assessed by the transfer index from blood into brain. Back-perfusion of the tracers was negligible because the tracer concentration in plasma exceeded that in brain tissue. Cortical blood flow in ischemic tissue was very low after MCA occlusion and did not change, as evidenced by $[^{14}]$iodoantipyrine autoradiography in the same model (unpublished data). On the other hand, brain plasma volume could affect the transfer index across capillaries despite intravascular perfusion. This brain:plasma ratio of radioisotope tracers is not a direct measurement of cerebrovascular permeability. We believe that the transfer index from blood into brain can determine relative changes in the permeability of the cerebrovasculature and comparatively at various intervals following an ischemic insult.

In our present study, extravasation of Evans blue and NaF1 was not evident in ischemic tissue ≤6 hours after MCA occlusion. The transfer index of the radioisotope tracers at this time did not increase significantly despite an increase in tissue water content. These findings are consistent with those of previous reports, which showed that BBB permeability to serum protein remains intact for at least several hours after an ischemic insult. $^{3,8,12}$ BBB permeability does not change directly with the accumulation of edema fluid. A similar conclusion resulted from our other studies, which showed that the early accumulation of edema fluid is associated with hydrostatic and osmotic pressure gradients.$^{4,5}$

A significant increase in BBB permeability was first found 12 hours after MCA occlusion. Staining by NaF1 was more extensive than that by Evans blue. The transfer index of $^{14}$C-sucrose increased gradually until water content reached a maximum 3 days after occlusion, whereas the transfer index of $^{125}$I-BSA remained at approximately the same level during this time, then increased markedly. There was a direct correlation between the accumulation of water and the increase in BBB permeability to $^{14}$C-sucrose. Movement of water into brain tissue could have been facilitated by the increase in cerebrovascular permeability to small molecules. Serum protein extravasated into ischemic brain may thus play a relatively minor role in the further accumulation of edema fluid. This suggestion contrasts with the more traditional concept that the extravasation of serum proteins introduces a vasogenic element and has a significant secondary effect on the formation of edema in ischemic brain tissue.$^{14}$ This idea is also supported by an experimental study showing that a pharmacologically induced opening of the BBB to serum protein does not increase water content in the brain tissue of rats.$^{22}$

After edema fluid accumulation peaked 3 days after occlusion, edema fluid resolved gradually. In contrast, BBB permeability to $^{125}$I-BSA and $^{14}$C-sucrose increased markedly during the same time and staining by NaF1 extended diffusely into ischemic brain tissue. Little is known about why BBB permeability changes maximally after ischemic edema has peaked. Some authors have observed histologically that the necrotic lesion is invaded by numerous macrophages containing cellular debris 7 days after MCA occlusion in cats.$^{18,22}$ At that time there is proliferation of the endothelium, with new vascular channels. Nonselective BBB permeability is probably related to passive leakage in necrotic vessels or through neovascular channels.

The pathophysiology of ischemic brain edema resolution is still poorly understood. One mechanism may be the uptake of extravasated proteins by glial cells, thus reducing the parenchymal oncotic force.$^{18}$ Another mechanism is biomechanical, shown by the migration of edema fluid that accumulates in the brain parenchyma through highly conductive tissue into the cerebrospinal fluid spaces.$^{6}$ In addition, our data indicate that resolution of edema fluid is associated with BBB opening. Therefore, reabsorption of edema fluid into the blood through an open BBB is one possibility for edema removal.

Early accumulation of edema fluid contributes to a hydrostatic pressure gradient, which develops soon after the onset of ischemia and is followed by the development of an osmotic pressure gradient. After these pressure gradients dissipate, further accumulation of edema fluid is associated with disruption of the BBB to small molecules. Extravasated serum protein does not appear to affect the accumulation of edema fluid. Resolution of edema fluid is probably related to an open BBB.

References


**KEY WORDS** • blood–brain barrier • brain edema • cerebral ischemia • rats
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