Microcirculatory Obstruction in Focal Cerebral Ischemia: An Electron Microscopic Investigation in Monkeys

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SUMMARY The fine structure of the microvasculature in areas of focal cerebral ischemia was studied in squirrel monkeys and the changes in areas of impaired and unimpaired microvascular filling, as defined by carbon perfusion, were compared. Microcirculatory obstruction became evident three hours following middle cerebral artery (MCA) occlusion and appeared to be partly the result of compression of capillaries by perivascular glial swelling and developing cerebral edema. Slight endothelial swelling was a common finding. Intraluminal membrane-bound bodies were occasionally identified but they did not appear to be producing significant obstruction. The tight endothelial junctions remained intact and there was no evidence of accelerated micropinocytosis. Severe neuronal injury frequently preceded the development of the microvascular obstruction and was more widespread than the zones of impaired perfusion.

The main steps in the procedure were as follows. (1) The right middle cerebral artery (MCA) in eight squirrel monkeys was occluded with a miniature aneurysm clip through a transorbital exposure. (2) Arterial blood samples (0.3 ml) were taken hourly for the determination of pH, Pao,, and Paco,. Rectal temperature and arterial blood pressure were constantly monitored. (3) Animals in groups of two were perfused after ischemic periods of 90 minutes, three hours, six hours, and 12 hours. They were initially perfused with 100 ml normal saline followed immediately by a mixture of colloidal carbon (250 ml) and phosphate-buffered 4% paraformaldehyde (250 ml). The clip was removed immediately prior to perfusion. (4) The brains were left undisturbed for two hours following perfusion. Then they were carefully removed from the skulls and placed in jars containing 50 ml phosphate-buffered 4% paraformaldehyde at 4°C for 24 hours. (5) The brains were cut coronally into 5 mm slices and the tissue was examined macroscopically. Thin (5 μ and 25 μ) coronal sections were prepared from paraffin-embedded slices of both hemispheres and stained with hematoxylin and eosin and with thionine. (6) Multiple specimens were taken from the gray and white matter of both hemispheres of each brain. Tissue was taken from the poorly stained, intermediate, and surrounding well-stained areas of those brains which had macroscopic evidence of impaired perfusion. It was postfixed in phosphate-buffered 1% osmium tetroxide for two hours and embedded in epoxy. The ultrathin sections were stained with uranyl acetate (2%) and lead citrate (0.1%).

References
Results

SYSTEMIC STABILITY

Arterial blood pressure remained stable and none of the animals became hypotensive. The pH, PaO₂, and PaCO₂ of the arterial blood remained within normal limits during occlusion.

MACROSCOPIC DISTRIBUTION OF COLLOIDAL CARBON

A detailed description of the carbon staining has been given in a previous report. Brains in the 90-minute group were well stained and there was no evidence of impaired microcirculatory filling or swelling. At three hours, both brains exhibited slight pallor of the right basal ganglia and the cortex in the distribution of the right MCA. One brain in the six-hour group was well stained resembling the brains subjected to only 90 minutes of ischemia. The other six-hour brain (fig. 1) and one of the 12-hour brains had a large cortical and subcortical area of poor staining. Swelling of the poorly stained regions was evident. A well-circumscribed poorly stained area involving the right lentiform nucleus and claustrum was present in the other brain in the 12-hour group.

LIGHT MICROSCOPIC FINDINGS

The light microscopic analysis of the material presented herein has been reported previously. In summary, no impairment of microvascular filling was detected with 90 minutes of occlusion and only mild impairment was detected with three hours of occlusion. Severe microcirculatory obstruction was present in zones of pallor at six and 12 hours. The obstruction appeared to lie at the capillary level,

FIGURE 1  Coronal section of monkey brain perfused with carbon-paraformaldehyde after six hours of right MCA occlusion. Note the large, pale area in the distribution of the right middle cerebral artery. Swelling of this area is grossly apparent. The gray matter of the left, nonischemic hemisphere is stained uniformly black. (R = right; L = left.)

FIGURE 2 A: At six hours in pale cortex, numerous erythrocytes (arrows) are packed into a recurrent capillary coming off a penetrating arteriole. Marked swelling of perivascular astrocytic processes and generalized cortical edema appear to be producing some narrowing of this vessel. The surrounding neurons are severely degenerated. B: At six hours in well-stained cortex, numerous capillaries containing carbon can be identified. A recurrent capillary (arrow) coming off a penetrating arteriole can be seen. There appears to be little reduction in the caliber of these vessels. The surrounding neurons are severely degenerated (scale: 50μ).
usually close to the feeding arterioles, and seemed partly the result of narrowing of the capillary channels by perivascular glial swelling and developing cerebral edema (fig. 2). Evidence of advanced neuronal injury, such as marked shrinkage, cytoplasmic eosinophilia, nuclear pyknosis and microvacuolation, were present in some regions at 90 minutes. Although severe neuronal changes were usually seen in areas of impaired filling, severe changes were invariably identified at an earlier stage than the impaired filling and in areas where perfusion did not appear to be impaired. Disrupted neurons were often seen lying adjacent to capillaries which were well filled with carbon.

ELECTRON MICROSCOPIC FINDINGS

Control Tissue

The fine structure of the microvasculature in the control tissue conformed to previous descriptions of the microvasculature in the normal central nervous system.\textsuperscript{3,4} Most of the vessels contained carbon particles (fig. 3A). Intraluminal clear spaces which appeared to be enclosed by a unit membrane were occasionally present.

Ischemic Tissue

Endothelium - mild to moderate endothelial swelling was noted at 90 minutes (fig. 3B) and was frequent at three hours. It seldom appeared severe enough to produce capillary obstruction; however, in the zones of pallor at six and 12 hours, the endothelial swelling occasionally reduced the lumen to a narrow slit (fig. 3C). Endothelial swelling in the intermediate and surrounding well-stained areas at six and 12 hours was relatively mild. Swelling of the mitochondria and endoplasmic reticulum was present at an early...
FIGURE 4 A: Longitudinal section of this capillary from the pale cortex at three hours demonstrates the perivascular distribution of the swollen glial processes (a). Endothelial swelling (e) also appears to be compromising the lumen somewhat. A normal-appearing pericyte (p) is present. B: No impairment of microvascular filling was detected in this section of basal ganglia at 12 hours. An encrusted neuron (n) and disrupted glia (g) lie in close proximity to a capillary which is well filled with carbon granules. There is no evidence of narrowing of the lumen. The cytoplasm of the associated pericyte (p) appears rarefied (scale = 3 μ).

stage. There was no evidence to suggest increased microsomal pinocytosis. The tight endothelial junctions (zonulae occludentes) maintained their integrity. Formation of so-called “blebs”6 from the plasma membrane lining the vessel lumen was not seen.

**Basement membrane** – the basement membrane appeared unaltered at 90 minutes. With longer periods of ischemia it occasionally had an irregular texture and was less dense. These changes were most prominent in the pale zones at six and 12 hours.

**Pericytes** – the main changes consisted of cytoplasmic rarefaction and swelling of the mitochondria and endoplasmic reticulum. Disruption of the pericytes was occasionally identified at 12 hours and no significant increase in the number of lysosomes occurred.

**Smooth muscle cells** – the smooth muscle cells in the arteriolar walls underwent a sequence of changes similar to the pericytes.

**Neuropil** – swelling of the perivascular astrocytic processes was noted at 90 minutes and became progressively more severe with longer periods of ischemia. Capillaries in the pale zones often appeared to be compressed by the layers of swollen processes surrounding them and the lumens of these vessels were frequently reduced to narrow slits (figs. 3D, E, and 4A). Although perivascular glial swelling was present in the intermediate and surrounding well-stained areas, it seldom appeared severe enough to compress the capillaries, and reduction in the size of the lumen was uncommon. Slight enlargement of the perivascular extracellular space was also seen in both the gray and white matter at 90 minutes. With longer periods of ischemia the edema appeared to spread in a centrifugal fashion involving regions most distant from the capillaries last. Disruption of the greatly swollen astrocytic processes at six and 12 hours contributed to the enlargement of the extracellular space.

**Neurons** – the evolution of ischemic nerve cell changes was similar to that described in previous investigations6–8 using the same experimental model. Evidence of severe neuronal injury, such as a marked increase in the cytoplasmic and nucleoplasmic density of shrunken neurons, disruption of limiting membranes, and loss of axosomatic synapses, was frequently present at 90 minutes. Advanced neuronal changes invariably were identified at an earlier stage than the impairment of carbon perfusion and were more widespread than the identifiable areas of impaired perfusion. Disrupted neurons were frequently seen lying adjac-
cent to almost normal-appearing capillaries filled with colloidal carbon (fig. 4B).

Intravascular compartment - the arterioles were generally well filled with carbon. Capillaries in the pale areas usually contained few carbon particles, whereas those in the intermediate and surrounding well-stained areas contained proportionately more carbon. At six and 12 hours, erythrocytes could be seen within capillaries where they usually appeared packed together (fig. 3F). Thrombi were infrequently identified. Clear spaces enclosed by a unit membrane (i.e., so-called "blebs") were occasionally seen. There was no evidence that they arose from the plasma membrane and they did not appear to be producing an obstruction.

Discussion

Electron microscopic examination confirmed the light microscopic findings in that the capillaries in areas of impaired filling appeared to be compressed by the swollen perivascular glial processes and developing cerebral edema. Capillary compression did not appear to be present in areas of unimpaired carbon perfusion. Although endothelial swelling was relatively mild in most instances, even slight reduction in the size of the capillary lumen produced by this mechanism is probably important in raising peripheral resistance and thereby interfering with tissue perfusion. Membrane-bound bodies were occasionlly present in the intravascular compartment of both the ischemic and control tissue. These structures corresponded to the so-called "blebs," and thought that they could play a role as an obstruction to flow. They suggested that these bodies originated from the endothelial plasma membranes and they did not appear to be producing significant obstruction.

Attention was first directed toward capillary compression as a possible cause of impaired microvascular perfusion by Chiang and associates following acute occlusion of the cerebral arteries and veins in the cervical region in rabbits. This observation was subsequently confirmed by Olsson and Hossmann in cats following temporary clamping of the major arteries supplying the brain. Endothelial swelling was also identified by both groups. Chiang et al. noted numerous membrane-bound intraluminal bodies, which they called "blebs," and thought that they could play a role as an obstruction to flow. They suggested that these bodies originated from the endothelial plasma membranes but their illustrations do not convincingly substantiate this hypothesis. Intraluminal blebs were rarely encountered by Olsson and Hossmann.

The fine structure of the microvasculature in areas of focal cerebral ischemia has been studied by Garcia and associates and Dodson and associates; however, neither group attempted to define areas of microvascular obstruction and compare the changes occurring within areas of impaired and normal filling. Garcia et al. observed that there was "no endothelial swelling or hyperplasia" and that "intraluminal blebs and capillary wall collapse were features not encountered." Dodson et al. failed to note the presence of endothelial swelling and capillary narrowing; however, their illustrations leave little doubt that these changes were present in their material.

Hemoconcentration, increased blood viscosity, and sludging of erythrocytes are factors which are also thought to contribute to the development of the microcirculatory obstruction. Blood viscosity has been shown to increase in areas of slow flow. Plasma skimming also appears to take place and is probably related to cellular aggregation and the selective obstruction of the cellular elements, mainly erythrocytes.

Ischemic edema developed in a centrifugal fashion beginning in the vicinity of the capillaries. The initial perivascular distribution of the astrocytic swelling suggested that it was related mainly to increased permeability. If the swelling of astrocytes was related primarily to depletion of energy reserves, one would expect it to occur in the areas most distant from the blood supply, provided the metabolic rate of astrocytes in different locations was similar. Enlargement of the extracellular space in gray matter may reflect a state wherein the astrocytes, unable to produce sufficient adenosine triphosphate (ATP) because of an inadequate oxygen supply, are incapable of metabolizing all of the fluid and electrolytes diffusing into the neuropil from the intravascular compartment.

The relationship between the distribution of the neuronal changes identified by light microscopy and the developing microcirculatory obstruction in the material presented here has been extensively described in a previous report. Electron microscopic examination confirmed the previously reported findings in that evidence of severe neuronal injury, such as the marked increase of cytoplasmic and nucleoplasmic density, disruption of cytoplasmic organelles, and fragmentation of membranes, frequently preceded the development of the microvascular obstruction and were more widespread than the zones of impaired microvascular filling. These findings suggested that neuronal injury is not necessarily the result of a microvascular obstruction as has been suggested by some investigators.

References

CEREBROVASCULAR DISEASE has been the most frequent cause of death in Japan since 1952. About half of the patients (90,000 every year) die from primary intracerebral hemorrhage, which usually occurs in elderly persons with arteriosclerosis and/or hypertension. However, very little progress has been made in the treatment of this disease in spite of the rapid advances in medicine during the last two decades.

The time has come for us to scrutinize this disease process and attempt to understand its pathology and pathophysiology. With the hope that surgical treatment may offer an effective approach to this illness, we began extensive studies on this subject at the Institute of Brain and Blood Vessels, Mihara Memorial Hospital, in 1966.

We have handled this disease as a major emergency in our stroke care units. Our surgical results, however, were as disappointing as those of McKissock et al.1-2 from an earlier period. With increasing experience, our surgical results are constantly being improved. We have concluded that surgical treatment is the most rational and effective therapy for primary intracerebral hemorrhage if it is performed on the basis of reasonably established indications.

In this paper we will propose a new angiographical classification system based on clinicopathological studies which, along with the patient’s level of consciousness, is felt to be the most important indication for operation.

**Methods**

Our study is based on 60 autopsy cases which had primary intracerebral hemorrhage in which ruptured arteries were examined by microangiography, the cleared specimens immersed in tetrahydrophthalene, and serial histological examinations performed between 1966 and 1968. We tried to find the arteries most commonly involved and the direction in which the hematoma advanced.

We analyzed 100 cases of primary intracerebral hemorrhage in which carotid angiography was carried out within two weeks after the onset and the diagnosis was confirmed at operation or autopsy during the same period. Hemorrhages due to other causes and verified by angiography, operation and autopsy were excluded.

**Results**

**PATHOLOGICAL FINDINGS**

**Ruptured Arteries (Figs. 1a and b)**

Figures 1a and b show normal microangiograms of perforating arteries supplying the putamen and thalamus, which are the most frequent sites of hemorrhage. There are three to six lateral lenticulostrate arteries that leave the trunk of the middle cerebral artery and enter the base of the...
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