Microcirculatory Obstruction in Focal Cerebral Ischemia: Albumin and Erythrocyte Transit

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SUMMARY The objectives were to study plasma and erythrocyte flow in an area of acute focal cerebral ischemia and define their relationship to developing microcirculatory obstruction as determined by morphological techniques. Eighteen adult cats, anesthetized with ketamine hydrochloride, had right middle cerebral artery (MCA) occlusion. Plasma flow was determined by measuring the transit of Iodine-131 ($^{131}$I) albumin and erythrocyte flow was determined by measuring the transit of Technetium-99 ($^{99}$Tc) labeled erythrocytes in the right Sylvian region. Transit studies were performed before and immediately after right MCA occlusion and at the end of the ischemic period, 1 hour, 3 hours, or 6 hours after occlusion. Arterial blood pressure was monitored continuously. A heating pad was placed over the trunk to keep the core temperature at 37°C. Arterial blood gases were determined when necessary to maintain the Paco$_2$ in the 30–35 torr range. Arterial blood pressure was monitored continuously. A heating pad was placed over the trunk to keep the core temperature at 37°C.

MICROCIRCULATORY CHANGES may play an important role in the evolution of a cerebral infarct. Much of the information regarding these changes has been derived from morphological and regional cerebral blood flow (rCBF) studies. These studies, however, have not answered important questions regarding the possible development of microcirculatory obstruction to the passage of erythrocytes or the separation of plasma and erythrocyte flow (i.e., plasmapheresis) during the early phases of cerebral infarction. The objectives of this investigation were to study plasma and erythrocyte transit in an area of acute focal cerebral ischemia and define their relationship to developing microcirculatory obstruction as determined by morphological techniques.

METHODS

Eighteen adult cats (mean weight 4.0 kg) were anesthetized with ketamine hydrochloride (40 mg/kg subcutaneously). Catheters were inserted into the right femoral artery and vein. A tracheostomy was performed through a longitudinal midline incision and mechanical ventilation instituted. Skeletal muscle paralysis was achieved with d-tubocurare (1.5 mg/kg IV). A small catheter was inserted into the right carotid artery through the lingual artery for subsequent injection of the isotopes. Arterial blood gases were determined when necessary to maintain the Paco$_2$ in the 30–35 torr range. Arterial blood pressure was monitored continuously. A heating pad was placed over the trunk to keep the core temperature at 37°C.

The head of each cat was shaved and fixed in a head holder which allowed unobstructed access to the right orbit. The orbital contents were evacuated and a 4 mm craniectomy, continuous with the superolateral margin of the optic foramen, was performed. Using microsurgical techniques, the dura and arachnoid membranes were opened. The proximal segment of the right middle cerebral artery (MCA) was carefully dissected from the adjacent structures in preparation for application of a miniature aneurysm clip. The scalp and temporalis muscle were then removed bilaterally from the skull overlying the Sylvian cortex.

Albumin and Erythrocyte Transit

An index of plasma flow was determined by measuring the transit of Iodine-131 ($^{131}$I) albumin in the right MCA territory. A collimated 1.5 cm sodium iodide crystal, recessed 5.0 cm, was applied to the skull overlying the Sylvian cortex. A second detector was placed on the skull overlying the left Sylvian cortex. The $^{131}$I window (364 keV) was determined with a multichannel analyzer. The $^{131}$I albumin (0.5 ml containing 100 μCi) was rapidly injected into the right internal carotid artery through the lingual artery catheter. Measurements were recorded on a strip chart recorder.

An index of erythrocyte flow was similarly determined by measuring the transit of Technetium-99 ($^{99}$Tc) labeled erythrocytes. Binding of the $^{99}$Tc to erythrocytes from a donor cat was carried out in vitro using the technique described by Schwartz and Kruger. Adequacy of the binding was determined by centrifuging 1 ml of the solution containing the $^{99}$Tc...
labeled erythrocytes and measuring the $^{99m}$Tc activity in the supernatant and packed erythrocytes using a well counter. Probe placement and measurement were similar to that used for the $^{131}$I albumin transit studies. The $^{99m}$Tc window (140 keV) was determined using a multichannel analyzer. The $^{99m}$Tc erythrocytes (i.e., 0.5 ml containing 100 $\mu$Ci) were rapidly injected into the right internal carotid artery through the lingual artery catheter. Measurements were recorded on a paper strip recorder.

Radionuclides were injected immediately before right MCA occlusion and again immediately after. The final injections were carried out at the end of the ischemic period, that is, 1 hour, 3 hours, or 6 hours after occlusion.

Six cats had $^{131}$I albumin transit studies only, these included 2 cats with 1 hour occlusion, 2 cats with 3 hours occlusion, and 2 cats with 6 hours occlusion. Six cats had $^{99m}$Tc erythrocyte transit studies only, these included 2 cats with 1 hour occlusion, 2 cats with 3 hours occlusion, and 2 cats with 6 hours occlusion. A group of 6 cats had both $^{131}$I albumin and $^{99m}$Tc erythrocyte transit studies including 2 cats with 1 hour occlusion, 2 cats with 3 hours occlusion, and 2 cats with 6 hours occlusion. In these animals, the $^{131}$I albumin and $^{99m}$Tc erythrocytes were injected sequentially with a 3 minute waiting period after the first injection.

**Perfusion Technique**

Thirty minutes before perfusion, sodium fluorescein (i.e., 0.5 ml of a 10% solution) and Evans blue (i.e., 0.5 ml of a 10% solution) were given intravenously. Intraarterial carbon-fixative perfusion was carried out at the end of the ischemic period after completion of the isotope studies. A midline thoracotomy was performed. The right MCA was reopened by removing the aneurysm clip. This was done to improve delivery of fixative to the ischemic tissue. A large cannula was passed through a left ventriculostomy incision into the ascending aorta and secured with a ligature. The descending aorta was clamped and the right atrium incised. The animals were perfused with 50 ml of isotonic saline followed by a mixture of colloidal carbon (250 ml) and phosphate-buffered formalin (250 ml) at a constant pressure of 120 mm/Hg. The brain of each cat was removed, sliced coronally, and placed in the fixative solution at 4°C for 48 hours.

**Examination of the Brains**

The coronal brain slices were photographed. The presence or absence of fluorescein and Evans blue was recorded. Shift of midline structures, if any, was measured.

The distribution and intensity of carbon staining was graded according to a previously-described system. Grade "0" indicated normal vascular filling; Grade "1" referred to a few circumscribed foci of poor filling, not more than 3 mm in diameter; Grade "2" indicated a large area of improper subcortical filling; and Grade "3" referred to an extensive cortical and subcortical region of impaired filling. The intensity of the zones of pallor were classified as mild, moderate, or severe (table).

The cerebral tissue under the detector probes was prepared for microscopic examination. A series of coronal sections (10 $\mu$ thickness) at 1 mm intervals were cut from paraffin-embedded slices, stained with hematoxylin and eosin and periodic acid Schiff stains, and examined with a light microscope. A total of 10 sections from each brain were examined.

Neurons were graded according to the severity of the changes present: Grade I — slightly shrunk or swollen neurons with or without cytoplasmic vacuolization; Grade II — moderately shrunk neurons with cytoplasmic eosinophilia and increased nuclear basophilia or swollen neurons with pale vacuolated cytoplasm and a pale vesicular nucleus; and, Grade III — severely shrunk neurons with bright cytoplasmic eosinophilia, pyknotic nucleus, and/or incrustations. The cross-sectional area of gray matter, where moderate and severe neuronal alterations (i.e. Grades II and III) predominated, were determined with a Keuffel and Esser planimeter in coronal sections of the right cerebral hemispheres 3 mm posterior to the temporal lobe tip. The percentage of gray matter surface area (i.e., ischemic gray area/total gray area X 100) where severe ischemic neuronal alterations predominated was determined.

Capillary luminal diameters were measured in the right and left Sylvian cortex, that is, the cortex underlying the detector probes. This was done using a calibrated eyepiece micrometer. Mean capillary luminal diameters were calculated. Measurement taken from the ischemic right Sylvian cortex were compared with those from the non-ischemic left Sylvian cortex using the Student's t-test.

**Results**

**Vital Signs**

Systemic stability was maintained in all cats. Mean systolic blood pressure was 95 ± 5 mm/Hg. Mean body temperature was 37 ± 0.5°C. Hematocrit was 34 ± 2%. Arterial blood gases were controlled by adjusting the ventilation. Mean $P_{aO_2}$ was 105 ± 5 torr and mean $P_{aCO_2}$ was 33 ± 2 torr.

**Morphological Studies**

1. **Macroscopic Findings.** Cerebral tissue in the right MCA territory was slightly swollen in 2 of the 6 cats.
after 1 hour occlusion. Four of 6 cats with 3 hours occlusion had slight to moderate swelling and shift of midline structures (i.e., ≤ 1 mm). Substantial swelling and midline shift (i.e., > 1 mm) were present in 5 of 6 cats after 6 hours occlusion (fig. 1).

Fluorescein staining was present in the caudate nucleus and/or cortex supplied by the right MCA of 1 cat after 1 hour occlusion, 4 cats after 3 hours occlusion, and 4 cats after 6 hours occlusion. In most instances the fluorescein staining was faint. However, in 1 cat after 6 hours occlusion, intense fluorescein staining was seen in the ischemic gray matter. Fluorescein was not detected in white matter. Evans blue staining was observed in the caudate nucleus and cortex in the right MCA territory of 3 cats after 6 hours occlusion.

The major branches of both right and left MCA’s were filled with the carbon solution. The distribution and intensity of carbon staining in the right hemispheres are recorded in the table.

2. Microscopic Findings. The evolution of ischemic changes was similar to previous detailed descriptions.6-8,10-17 Grade I and II neuronal alterations were seen in the caudate nucleus and/or cortex supplied by the right MCA of 6 cats after 1 hour occlusion. These neuronal changes invariably had a multifocal distribution. Severe neuronal alterations (i.e., Grades II and III) were present in both caudate nucleus and cortex in 4 cats after 3 hours occlusion and 5 cats after 6 hours occlusion. Ischemic neuronal changes were not present in the cortex of 2 cats in the 3 hour group and 1 cat in the 6 hour group. Mean percentage of gray matter cross-sectional area with severe neuronal alterations was 15 ± 5% at 1 hour, 30 ± 10% at 3 hours and 55 ± 12% at 6 hours.

Mild to moderate pericapillary and perineuronal vacuolation were seen in cortex and/or caudate nucleus after 1 hour of MCA occlusion. At 3 hours, vacuolation also was present in the neuropil and in the periarteriolar and perivenous regions. The most severe changes were present in 5 after 6 hours occlusion. Ischemic edema invariably was restricted to areas where neuronal alterations were present.

Mean capillary luminal diameter in the non-ischemic left Sylvian cortex was 6.5 ± 0.5 μ. Progressive capillary luminal narrowing was seen in the right Sylvian cortex of cats with findings of ischemic edema and neuronal changes. Mean capillary luminal diameter in these cats was 6.0 ± 0.5 μ at 1 hour (p < 0.01), 5.5 ± 0.5 μ at 3 hours (p < 0.01), and 5.0 ± 1.0 μ at 6 hours (p < 0.001). Capillary luminal narrowing was not seen in right Sylvian cortex of those cats without other ischemic changes.

Isotope Transit Studies

The transit time referred to the time required for a single passage of the isotope-labeled material through the cerebral circulation underlying the detector probe. This was determined from measurements of the total peak time.131I albumin transit time in the right Sylvian region was 8 ± 2 seconds before occlusion (fig. 2A). It increased to 10 ± 2 seconds immediately after right MCA occlusion (fig. 2B). The peak of the transit curve after occlusion had a rounder contour than before occlusion. With 1 hour occlusion, transit time was 13 ± 1 seconds (fig. 2C). In the cats with an ischemic cortical lesion, transit time was 15 ± 2 seconds after 3 hours occlusion and 19 ± 3 seconds after 6 hours occlusion (figs. 2D and E). In 2 cats without morphological changes in the right Sylvian cortex, one after 3 hours occlusion and another after 6 hours occlusion, there was no prolongation of transit times when compared with the immediate post-occlusion studies (fig. 2F). Peak amplitudes when present on the left side were less than 10% of those recorded by the right-sided detector.

99mTc erythrocyte transit time in the right Sylvian region was 10 ± 2 seconds before occlusion (fig. 2A). The transit time increased to 12 ± 3 seconds immediately after right MCA occlusion (fig. 2B). The peak of the transit curve after occlusion had a rounder contour than before occlusion, similar to that seen with the 131I albumin transit studies. After one hour occlusion, transit time was 15 ± 3 seconds (fig. 2C). In those cats with an ischemic cortical lesion, transit time was 16 ± 1 seconds after 3 hours occlusion and 20 ± 2 seconds after 6 hours occlusion (figs. 2D and E). In all cats, the counts returned to a baseline or near baseline position after the initial transit. In 2 cats without morphological changes in the right Sylvian cortex, one after 3 hours occlusion and another after 6 hours occlusion, there was no prolongation of transit time when compared with the immediate post-occlusion studies (fig. 2F). Peak amplitudes when present on the left side were less than 10% of those recorded by the right-sided detector.

Transit times for 99mTc erythrocytes in the combined injections were slightly longer than the transit times for 131I albumin in all but one experiment where the transit times for 99mTc erythrocytes and 131I albumin were essentially the same.
The erythrocyte fraction was found to contain 85 ± 3% of the 99mTc activity.

Discussion

Microcirculatory obstruction (i.e., "no-reflow phenomenon") was initially demonstrated in ischemic brain by Ames et al. Ischemia was produced in rabbits by sectioning the basilar artery, clamping the common carotid arteries, and constricting the neck with a pneumatic cuff. Large perfusion defects, demonstrated by the colloidal carbon perfusion technique, were consistently seen with 15 min occlusion.

Subsequent morphological studies in primates and cats undergoing MCA occlusion have provided further insight into the development of microcirculatory impairment and its relationship to changes in the parenchyma. The ischemic lesion was found to develop in a diphasic fashion. Microcirculatory obstruction, as demonstrated by carbon perfusion, did not occur until the secondary phase, that is, at a time when irreversible changes had already occurred. However, important changes, such as capillary and venous narrowing, capillary separation, and endothelial swelling seen during the primary phase, were thought to be important factors in impairing microcirculatory flow.

Erythrocyte sludging in the microvasculature also appeared to be an important factor. Rheologic studies performed by Merrill have shown that blood viscosity increases as the rate of flow decreases. Increased viscosity was attributed to erythrocyte aggregation and was found to be a function of the third power of the hematocrit. Fischer and Ames
subsequently demonstrated substantial reduction of impaired carbon filling in the rabbit model of global brain ischemia by acute hemodilution with saline. Their findings appeared to substantiate the importance of blood viscosity changes.

Cerebral blood flow following acute experimental MCA occlusion in primates and cats has been measured by a variety of techniques including diffusible isotope clearance (i.e., hydrogen, Xenon, Krypton), cerebral autoradiography and microsphere injection. These studies have consistently demonstrated substantial rCBF reduction immediately following MCA occlusion. The most severe, persistent reduction in rCBF in the occluded MCA territory of cats was reported by Hossmann and Schuier using 15 µ radioactive microspheres. Regional CBF initially dropped to 9.2 ± 2 ml/100 gm/minute (control: 32 ± 4 ml/100 gm/minute) in cats developing a morphological lesion and fell to lower levels during the subsequent 4 hours. This secondary rCBF decline appeared to correlate with the progressive brain swelling seen in this group of animals. Unfortunately, MCA occlusion longer than 4 hours was not studied.

The pre-occlusion transit times for 181I albumin (i.e., 8 ± 2 seconds) and 99Tc erythrocytes (i.e., 10 ± 2 seconds) were similar to previously-reported values for normal brain. Occlusion of the MCA resulted in an immediate 2 second delay of the transit times in the right Sylvian region. Additional increases in transit times occurred with longer periods of ischemia in cats developing morphological changes in the Sylvian cortex. This progressive impairment of the microcirculation correlated with the increasing ischemic edema and capillary luminal narrowing. In those cats without morphological change or with changes restricted to subcortical structures (i.e., caudate nucleus), transit times at the end of the occlusion period were essentially unchanged from those immediately after MCA occlusion. These findings, together with the low counts recorded over the non-ischemic left hemisphere, indicated that the information recorded by the right-sided detector was derived primarily from cortex.

Microcirculatory obstruction, as demonstrated by carbon perfusion studies, probably does not represent a state of complete microvascular occlusion. Ames et al. in their initial description of the so-called "no-reflow phenomena," eliminated many of the carbon perfusion defects by washing out the brain circulation with Ringer's solution prior to the onset of ischemia. Olson and Hossmann made similar observations. Subsequent studies by Fischer and Ames revealed that the amount of obstruction demonstrated by carbon perfusion was inversely related to the infusion pressure. In our study, no evidence of complete microcirculatory obstruction to albumin and erythrocyte transit was seen in cats undergoing 6 hours of MCA occlusion despite the impaired filling of the cortical microcirculation with carbon.

Rosenblum postulated a state of "plasma skimming" (i.e., plasmapheresis) developing in brain ischemia. His conclusions were based largely upon direct observations of the cortical microcirculation in anoxic mice. He expressed concern that "plasma skimming" or "inconsistency of regional hematocrit" could produce serious errors in rCBF measurements using diffusible tracer techniques. Subsequent morphological studies in primates and cats undergoing MCA occlusion also suggested a separation in plasma and erythrocyte flow (i.e. plasmapheresis).

In the present study, albumin transit was slightly more rapid than erythrocyte transit. This relationship remained constant both before and after MCA occlusion in the cats undergoing combined isotope injections. There was no evidence of disproportionate delay of erythrocyte transit as compared with plasma transit during the evolution of infarction. This indicated that a state of plasmapheresis had not developed. However the limitations of the measurement technique used could obscure the presence of plasmapheresis within the small areas of the cortex which were studied.

The relationship of the early microcirculatory changes to subsequent tissue viability has not been clearly defined. Studies directed toward the prevention of microcirculatory impairment, however, suggest that microvascular changes play an important role. Administration of hyperosmolar agents, such as mannitol and low-molecular-weight dextran, have been shown to suppress the development of ischemic edema and microvascular changes and thereby delay the onset of ischemic neuronal injury. These findings indicate that treatment of acute focal cerebral ischemia should be directed towards the maintenance of the microcirculation as well as the preservation of neuronal metabolic integrity.

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