Cerebral Blood Flow Determined by Hydrogen Clearance During Middle Cerebral Artery Occlusion in Unanesthetized Monkeys


SUMMARY Unanesthetized macaque monkeys were subjected to middle cerebral artery (MCA) occlusion. Local cerebral blood flow (ICBF) was measured with a hydrogen clearance technique. Mean arterial blood pressure, arterial blood gases, and intracranial pressure were monitored serially. Two weeks after ischemic insult, a neuropathologic examination documented cerebral infarction and its relation to CBF recording sites.

Unanesthetized monkeys hyperventilated and became hypocapneic, particularly after MCA occlusion (mean Paco2 fell from 33 to 26 mm Hg). Intracranial pressure remained normal except in massive fatal infarction, where it rose dramatically.

Responses to MCA occlusion were strikingly variable; some animals showed little ICBF reduction and no deficit or infarction; while others sustained marked ICBF reduction with hemiplegia and massive fatal infarction. Extent of ICBF reduction correlated well with infarct size. Since the extent of ICBF reduction reflects collateral circulation, variation in collateral supply appears responsible for variation of infarction.

Re-opening of MCA occlusion always led to restoration of normal or above normal ICBF. Thus, no evidence for impaired reperfusion was observed. Post-ischemic CBF markedly above normal was associated with eventual severe infarction. Restoration of MCA flow led to improvement of hemiparesis after moderate reduction in ICBF, but fatal infarction occurred despite restored flow after severe reduction in ICBF. "No reflow" was not observed.

When ICBF fell below 12 cc/100 gm/min for 2 hours or longer, local infarction resulted. Flow above this infarction threshold prevented irreversible damage.

IMPROVED THERAPY for ischemic strokes will depend on enhanced understanding of their pathophysiology. In particular, the defining characteristics of irreversible damage need to be identified, since treatment will be most useful only before reversible changes become permanent. Some function of cerebral blood flow (CBF) reduction and its duration probably defines a threshold for irreversible infarction. In turn, the extent of CBF reduction after cerebrovascular occlusion likely reflects the availability of collateral circulation.

An animal model offers the best opportunity to examine the CBF threshold for irreversible damage after cerebrovascular occlusion. To be most useful, such a stroke model must (1) use a cerebrovascular system similar to that of man, (2) avoid the unphysiologic effects of anesthesia, (3) monitor ischemia-modifying variables such as blood pressure and blood gases, (4) monitor sequential changes in local CBF following occlusion, and (5) assess ischemic change by neurologic examination and irreversible damage by morphologic evaluation.

We report here a pilot study in an animal model designed to meet these criteria. In unanesthetized Macaca irus monkeys, the middle cerebral artery (MCA) was occluded temporarily. Arterial blood pressure and blood gases were monitored. Serial measurements of local CBF (ICBF) were made at 6 sites by hydrogen clearance. Neurologic status was examined sequentially. Neuropathologic evaluation was performed 2–3 weeks after MCA occlusion to identify infarction and its relation to sites of ICBF recording.

Results suggest the model is useful. Responses to MCA occlusion varied strikingly, but extent of ICBF reduction correlated well with infarct size. A threshold for infarction was identified: when ICBF fell below 12 cc/100 gm/min for 2 hours or longer, local infarction occurred.

Methods

We studied 8 Macaca irus monkeys, unscreened for sex and weighing 3.5–4.5 kg. A preparatory operation was performed under general anesthesia to install an aortic catheter, platinum electrodes for measurement of ICBF, epidural transducers for measurement of ICP (intracranial pressure), and a ligature around the right middle cerebral artery. After surgery, the animal was placed in a standard primate restraining chair. The following day, baseline data were gathered, and the MCA ligature was tightened for intervals of 120, 150 or 180 minutes (permanent occlusion in 1 case) to produce temporary occlusion. Before and during occlusion and after reocclusion, we monitored mean arterial pressure, arterial blood gases, neurologic status, ICBF, and intracranial pressure. ICBF was monitored immediately after reocclusion, and at 24, 48, 72 hours, and 7, 10 and 14 days. In some cases late measurements were made. Forty-eight to 72 hours after occlusion the monkey was returned to its cage. Neuropathologic evaluation utilizing horizontal serial sections identified infarcts and their relation to ICBF recording sites.

Technique of MCA Occlusion

Under phencyclidine anesthesia (Bio-ecute Laboratories, Inc., St. Joseph, MO) Macaca irus monkeys underwent...
right orbital exenteration and posterior orbital craniectomy for exposure of the carotid bifurcation. 10 Using the operating microscope, a 10-0 nylon snare ligature was passed around the right MCA at its origin and brought through the orbit via a PE-10 polyethylene catheter (Clay Adams, Parsippany, NJ). The suture was then tied around an additional short segment of PE-10 catheter, and the amount of traction necessary for occlusion was determined. 11 The orbit was sealed with acrylic (Caulk Grip Cement, L.D. Caulk Co., Milford, DE) in watertight fashion, and periorbital tissues were reapproximated. Twenty-four hours later 6 monkeys were subjected to focal cerebral ischemia, and an additional 2 animals served as controls.

Local Cerebral Blood Flow

ICBF was measured using a hydrogen clearance technique. 11 12 Electrodes were made of 70% platinum 30% iridium wire (Medwire Co., Mt. Vernon, NY). 0.10 inch in diameter and Teflon-coated save for a 2mm bare tip. An array of electrodes was placed stereotactically in the area known to be ischemic after MCA occlusion. 13 Five electrodes were implanted in the right posterior frontal region with a sixth control electrode in the left posterior frontal region. Electrode number 1 was placed in the globus pallidus using stereotactic technique, and electrodes 2 through 5 were placed at 2.5-3 mm intervals moving laterally in the right hemisphere (see fig. 5). Electrode 6 (control) was placed in the left hemisphere. A reference electrode was placed in the anterior frontal skull. Extracranial microconnectors were imbedded in acrylic and fixed to the skull; this arrangement allowed the monkey to be returned to his cage between determinations. Hydrogen gas (about 4%) was fed into a plastic breathing chamber placed over the monkey's head. A vacuum venting system permitted prompt evacuation of hydrogen from the chamber at the cessation of hydrogen inhalation. Electrodes were polarized at +0.65 volts according to a modification of the voltage clamp circuit of Willis et al. 19 The first 40 seconds of hydrogen washout were disregarded to avoid problems of recirculation. 19

Other Monitoring Methods

Intracranial pressure (ICP) measurements were made using a modification of the epidural pressure transducer system described by O'Brien and Waltz. 14 In each parietal region an epidural pressure transducer was placed coplanar to the dura and fixed to the skull. A pressure in the epidural space of 0 mm of mercury was assumed for the normal monkey in the upright position. Statham® Model P-23 transducers (Gould-Statham,® Hato Rey, PR) were used to measure pressures. A femoro-aortic arterial line allowed monitoring of mean arterial pressure and arterial blood gases. A Grass polygraph (model 7B, Grass Medical Instruments, Quincy, MA) recorded ICBF, blood pressure, and ICP.

Observations During Focal Ischemia

On the day following preparatory surgery, we carried out a neurological examination on the monkey in a restraining chair. Baseline determinations were made for ICBF, ICP, mean arterial pressure and arterial blood gases. MCA occlusion was then achieved by applying traction to the snare ligature; traction was maintained with aneurysm clips. After 120-180 minutes of occlusion, ischemia was reversed by releasing traction on the snare ligature. (A single animal underwent permanent MCA occlusion.)

Neuropathology

Monkeys which did not expire acutely were sacrificed 2-3 weeks after the onset of the ischemic insult. Monopolar cauterization was applied to each electrode to help in identification of electrode tip sites. Brains were fixed in situ by trans-cardiac perfusion with 10% phosphate-buffered formalin. The right internal carotid artery was catheterized in the neck and injected with Microfil® to document patency of the right MCA at the point of temporary occlusion. In every case the vessel was patent with the exception of the permanently occluded vessel. The brains were removed and immersed in the perfusion fixative for an additional 2 to 3 weeks. The brain stem was then severed at the midbrain and the entire brain was embedded in celloidin. The whole brain was then serially sectioned in the horizontal plane in 15 micron sections. The sections were sequentially identified and stained in the following fashion: the first 8 sections were segregated in a "stain bottle" and the subsequent 17 sections were labelled "save." Sections from the "stain bottle" were stained with hematoxylin and eosin, cresyl violet and Loyez stain for myelin. The procedure was repeated for the entire brain so that, in effect, sections available for microscopic review were at 3.75 millimeter intervals. Sections were examined sequentially to locate the electrode tracts and tips (cauterized tissue) and to identify size, location, and character of infarction in relation to the electrodes.

Results

Systemic Factors

These monkeys showed variable levels of alertness, ranging from calm to agitated with vocalization and tail movements. Moderate hypertension (cf. table 1) was probably related to agitation. Likewise, agitation caused hyperventilation and a persistent alkalotic hypocapnea, which was more pronounced during and after MCA occlusion. PaCO2 measurements demonstrated normal oxygenation for all animals. Hematocrits tended to fall gradually

Table 1 Summary of Blood Gases, Blood Pressures and Hematocrits Before, During and After MCA Occlusion (where applicable). Mean Values with Standard Deviations

<table>
<thead>
<tr>
<th></th>
<th>Before occlusion</th>
<th>During MCA occlusion</th>
<th>After MCA occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>121</td>
<td>122</td>
<td>110</td>
</tr>
<tr>
<td>±12.8 (norm)</td>
<td>±14.7 (norm)</td>
<td>±9.6 (norm)</td>
<td></td>
</tr>
<tr>
<td>Pao2 (mm Hg)</td>
<td>90.4</td>
<td>93.4</td>
<td>89.0</td>
</tr>
<tr>
<td>±15.5 (norm)</td>
<td>±9.6 (norm)</td>
<td>±11.7 (norm)</td>
<td></td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>33.0</td>
<td>26.0</td>
<td>28.3</td>
</tr>
<tr>
<td>±5.8 (norm)</td>
<td>±6.4 (norm)</td>
<td>±5.0 (norm)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.43</td>
<td>7.52</td>
<td>7.44</td>
</tr>
<tr>
<td>±.04 (norm)</td>
<td>±.04 (norm)</td>
<td>±.04 (norm)</td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>30.0</td>
<td>28.5</td>
<td>26.1</td>
</tr>
<tr>
<td>±1.0 (norm)</td>
<td>±4.6 (norm)</td>
<td>±3.6 (norm)</td>
<td></td>
</tr>
</tbody>
</table>
over the study period (from a mean of 30% down to 26%), probably related to frequent arterial blood gas sampling.

**Local CBF Studies**

ICBF studies generally produced monoexponential washout curves, with occasional biexponential exceptions. Baseline ICBF values for electrodes in basal ganglia or insula ranged from 34 to 101 cc/100g/min. Serial values of ICBF from control electrodes varied up to ± 15% over the 2-3 week survival period.

**Response to MCA Occlusion**

MCA occlusion led to a neurologic deficit within 3 minutes in all 6 animals. Five of the animals developed a complete left facial paresis, flaccid left upper limb distal to the shoulder, markedly paretic left lower limb, with turning of head and eyes to the right and unresponsiveness to threat or food presented from the left. In 1 animal, MCA occlusion produced only mild upper limb paresis. MCA occlusion led to an immediate decrease in ipsilateral ICBF in all animals (table 2, figs. 1-3). Residual flow in the most lateral ICBF in all animals (table 2). Residual flow at individual electrodes ranged from 9% to 107% of pre-occlusion values (2-3 determinations) remained stable. In the single experiment #5 in which a hemorrhagic infarct was found, and Experiment #4, where acute ischemic infarction was present. Histopathological changes were typical of ischemic infarcts of 2 weeks duration (fig. 5), with the exception of Experiment #5 in which a hemorrhagic infarct was found, and Experiment #4, where acute ischemic infarction was present. Infarcts varied in size and location: Experiment #1 showed

**Response to MCA De-occlusion**

After de-occlusion at 2-3 hours, 3 animals showed prompt neurologic improvement, readily discernible within 30-60 minutes. Two monkeys (#3 and #6) with severe ischemia (ICBF less than 15 ml/100g/min) improved promptly to a moderate hemiparesis with further gradual improvement up to the time of sacrifice (fig. 2). One monkey (#1) with moderate ischemia (ICBF more than 15ml/100g/min) made an immediate full recovery (fig. 1). After de-occlusion in these animals, ICBF rose to levels above normal at most electrodes and ICP remained normal.

Two monkeys (#4 and #5) with profound focal ischemia (less than 5ml/100g/min) showed no improvement after de-occlusion but instead developed progressive drowsiness with death at 24 and 72 hours (fig. 3). These were the only monkeys sustaining slight subarachnoid hemorrhage during ligature placement. Both animals demonstrated marked reactive hyperemia, which appeared immediately in 1 and at 24 hours in the other. The latter monkey demonstrated a marked terminal rise in ICP (> 1000 mm water). Animals not expiring acutely (#1-3 and #6) showed no rise in ICP.

**Neuropathologic findings**

Serial horizontal sections through all the brains allowed precise mapping of the electrode tracts (fig. 5). Distinguishing between infarcted tissue and cauterization necrosis was sometimes difficult — impossible in 1 animal that died acutely (#4). In general, electrode 1 lay in the internal capsule, 2 in the putamen, 3 and 4 in the insular white matter, 5 in the insular cortex and 6 in the contralateral putamen (table 2). Histopathological changes were typical of ischemic infarcts of 2 weeks duration (fig. 5), with the exception of Experiment #5 in which a hemorrhagic infarct was found, and Experiment #4, where acute ischemic infarction was present. Infarcts varied in size and location: Experiment #1 showed

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**TABLE 2 Summary Chart Showing Duration of Occlusion, Clinical Findings, Electrode Site, Flow During MCA Occlusion, and Pathology for Each Monkey**

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Duration of ischemia (min)</th>
<th>Clinical response</th>
<th>Electrode Site</th>
<th>ICBF and Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>Hemiparesis after de-occlusion</td>
<td>Site*GP P I I CX</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Permanent</td>
<td>Transient mild hemiparesis, complete resolution</td>
<td>Site AIC P P I CX</td>
<td>40 27 36 38 43</td>
</tr>
<tr>
<td>3</td>
<td>Hemiparesis during occlusion, partial resolution</td>
<td>Site AIC P I I CX</td>
<td>26 40 31 49 43</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>123</td>
<td>Hemiplegia, no improvement; death at 72 hrs.</td>
<td>Site AIC P P P CX</td>
<td>9 4 3 3 3</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>Hemiplegia, no improvement; death at 72 hrs.</td>
<td>Site AIC P P CX</td>
<td>15 14 5 5 13</td>
</tr>
<tr>
<td>6</td>
<td>157</td>
<td>Hemiplegia, partial resolution</td>
<td>Site AIC I I CX</td>
<td>10 8 10 42 0</td>
</tr>
</tbody>
</table>

Abbreviations: GP = globus pallidus; P = putamen; I = insular white matter; CX = insular cortex and AIC = anterior limb of internal capsule; hem = hemorrhage.
an infarct 50–75 microns in diameter in the deep gray matter of the globus pallidus distant from the electrode tips. Experiment #2 showed an hourglass-shaped infarct in the anterior limb of the internal capsule and deep medial globus pallidus. This 100 micron infarct came close to electrode 1 but did not touch it. Experiment #3 showed an infarct less than 1 cm in diameter involving electrodes 2, 3, and 4. Experiment #4 showed acute infarction of the entire territory of the middle cerebral artery with massive edema and poor localization of the electrode tips. Experiment #5 showed a 2 cm hemorrhagic infarct with extension to insular cortex and poor electrode localization. Experiment #6 showed a 1.5 cm. ischemic infarct surrounding electrodes 1 and 2 and adjacent to electrode 3.

Physio-pathologic Correlations

ICBF during MCA occlusion correlated closely with infarct size. The greater the residual ICBF (which can only come from collateral circulation), the smaller the infarct (see table 2). In animals with good collateral circulation (#1 and #2, fig. 1), 2 hours and permanent occlusion led to little or no infarction. In animals with moderate collateral (#3 and #6, fig. 2), 2–3 hour occlusion caused medium-sized infarction. In animals with poor collateral (#4 and #5, fig. 3), 2 hour occlusion produced massive fatal infarction.

When ICBF during MCA occlusion remained above 12cc/100g/min, tissue around the recording electrode showed no infarction (see fig. 4). (Two exceptions involved electrodes which lay on the edge of an infarction and thus may have recorded higher flow from nearby preserved tissue.) When residual ICBF fell below 12cc/100g/min for 2 hours or more, infarction invariably occurred around the electrode tip. In our animals, the infarction threshold was about 30% of pre-occlusion ICBF. No definite correlation could be drawn between hemorrhagic infarction and ICBF values.
Unanesthetized Monkey Model

Our unanesthetized monkey preparation presents several advantages for the study of focal cerebral ischemia. This primate species simulates closely the human cerebrovascular system, and the snare ligature technique eliminates the confusing effects of general anesthesia. This snare ligature method uniquely permits precisely reproducible temporary MCA occlusion. Ischemia-modifying factors can be readily monitored in this preparation, and neurologic examinations can be performed.

The most notable deviations from normal in these animals were the hypertension and hypocapnic alkalosis induced by variable hyperventilation. In this sense, the unanesthetized state is itself a variable for such cerebrovascular studies. It may be possible to limit agitation by conditioning monkeys to the restraining chair and strictly limiting sensory stimulation. Another deviation from normal was the declining hematocrit, probably resulting from repeated blood gas sampling. Monitoring of end-tidal CO₂ and placement of intraarterial oxygen electrodes may decrease need for blood sampling and thus eliminate the fall in hematocrit.

Local Cerebral Blood Flow

The values of ICBF recorded here in basal ganglia are less than those reported by others. This is likely related to chronic hyperventilation in these awake monkeys and to the large size of our electrodes (2mm exposed tip). Some of the variation in ICBF recorded from a single electrode may be related to changes in alertness. The monoexponential character of most wash-out curves suggests a restricted locus of...
Electrode

FIGURE 3. Poor collateral supply — massive fatal infarction. Left: (Monkey §5): Marked hemiparesis during 2 hour profound ischemia. Fatal course with marked hyperemia after deocclusion, massive hemorrhagic infarction. Right: (Monkey §4): Marked hemiparesis during 2 hour profound ischemia. Fatal course with delayed marked hyperemia after deocclusion; massive infarction. Note that only monkeys §4 and §5 had subarachnoid hemorrhage during ligature placement.

FIGURE 4. Infarction threshold. Pooled data from 6 monkeys. Open circles stand for electrodes lying in preserved tissue, black squares are electrodes lying in infarcted tissue, and half-black squares are electrodes on the edge of infarction. Note the infarction threshold at about 12cc/100gm/min.

blood flow monitoring. That the electrodes indeed determine flow in largely uninjured tissue is suggested by the relative stability of ICBF values over time, the preservation of autoregulation and CO₂ responsivity and the limited histological reaction.

Response to MCA Occlusion

As in the cat, 4 MCA occlusion caused immediate deficit and ischemia. Residual ICBF generally stayed constant during 2–3 hour MCA occlusion; that is, early failure or enhancement of collateral was not seen. 4 Diaschisis was not observed; in fact, most animals showed contralateral hyperemia during occlusion.

Slight subarachnoid hemorrhage during preparatory surgery occurred in 2 animals and was associated in both with profound ischemia and infarction. Vasospasm, though asymptomatic before occlusion, may well have compromised the collateral supply in these animals. We suspect that massive infarction may have been a byproduct of unwanted surgical subarachnoid bleeding, not of a pre-existing paucity of collateral supply.

Response to De-occlusion

Since MCA de-occlusion always led to normal or hyperemic ICBF, no evidence for impaired reperfusion 4 was
observed in these experiments. In 3 animals with moderate ischemia, restoration of flow led to improved CBF and neurologic status, but in 2 monkeys with profound ischemia, restoration of flow was probably deleterious (cerebral edema and hemorrhagic infarction, fig. 3). Marked reactive hyperemia heralded infarction, but in 1 case moderate reactive hyperemia was associated with full recovery.

Physiologic-Pathologic Correlations

The close correlation of residual CBF from collateral with infarct size supports the concept that variability in strokes is related to variable collateral supply. In these experiments, infarction occurred in unanesthetized monkey brain after MCA occlusion if CBF fell below 12cc/100g/min for 120 to 180 minutes. This infarction threshold corresponds roughly to the level of about 10cc/100g/min below which substantial release of K⁺ occurs in the brain. Ischemic injury probably is related to the time of exposure to a level of ischemia and the duration of exposure to that level of ischemia. Therefore, infarction requires lower flow values if exposure time is short. Further experiments will be needed to establish this point. Conceivably, such an infarction threshold could be used to diagnose irreversible damage and aid selection of cases for surgical revascularization. A threshold for temporary functioning derangement is likely significantly higher. When CBF falls below 17–18cc/100g/min in unanesthetized humans, major abnormalities appear.

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References


Figure 5. Horizontal sections of brain. Loyez stain for myelin. A. Experiment §3. Infarced tissue is outlined by thick arrow heads. Electrode tracts seen as holes are numbered 1–6. Electrodes 1 and 2 are within the infarcted zone, electrode 3 borders the infarct, and electrode 6 is the control electrode in the putamen contralateral to the occluded middle cerebral artery. B. (Inset) Experiment §2. Electrode tracts 1–5 in non-infarcted tissue.
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