
Differential Regional Vulnerability in Transient Focal Cerebral Ischemia

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SUMMARY An unanesthetized monkey model for transient focal cerebral ischemia was used to determine differential regional vulnerability, as defined by histologic criteria for injury. Awake Macaque monkeys underwent unilateral, temporary middle cerebral artery (MCA) occlusion for a period ranging from 15 minutes to 3 hours. Hydrogen clearance was used to monitor local cerebral blood flow (LCBF) from affected cortical and subcortical sites before, during, and after occlusion. Two to four weeks following MCA occlusion, animals were sacrificed and neuropathologic evaluation identified cerebral tissue damage and its precise relation to blood flow recording sites.

MCA occlusion led to decreased ipsilateral flow and neurological impairment in all animals. Decreases in LCBF were largest in putamen. MCA de-occlusion led to recovery of neurological function in some, but not all, animals and return of LCBF to near control in all surviving animals. Neuropathologic evaluation revealed areas of incomplete, selective tissue necrosis in the gray matter and areas of total necrosis in both gray and white matter. Selective necrosis was associated with brief, moderate ischemia, while total necrosis was associated with longer, more severe ischemia. Tissue damage was observed both earlier and at higher levels of LCBF in the gray matter than in the white matter. Post-ischemic flow levels during the weeks following occlusion did not correlate with either the degree of residual neurological impairment or the extent or severity of cerebral tissue damage. The data indicate gray matter is more vulnerable to a given ischemic insult than white matter.

WHEN A MAJOR CEREBRAL ARTERY is abruptly occluded, many factors play a role in determining whether damage to the brain will occur. The adequacy of collateral circulation governs the severity and extent of ischemia. The duration of ischemia is critical for recovery of function. Blood flow thresholds of ischemia have been defined for paralysis and infarction. For cerebral ischemia of a given degree, some areas may be especially vulnerable. Zones of greater metabolic activity may require more substrate to maintain structural integrity. Thus gray matter, with its greater metabolic activity, might be expected to be particularly sensitive to ischemia. Some histopathologic studies have supported this notion, but correlated blood flow data have been lacking.

We have investigated regional cerebral vulnerability...
to ischemia in a primate model of focal cerebral ischemia. Unanesthetized monkeys were subjected to temporary snare ligature occlusion of one middle cerebral artery (MCA) during registration of local cerebral blood flow (LCBF). Histopathological examination assessed tissue damage which was correlated with blood flow data. Preliminary results have indicated gray matter is more vulnerable to a given ischemic insult than white matter.

Materials and Methods

Nineteen monkeys (Macaca fasicularis) were used in this study. Animals weighed 3.5 to 7.5 kg and were housed individually in an animal care facility with a 12 hour on and 12 hour off light-dark schedule. Phenylephrine hydrochloride (1 mg/kg) or Ketamine hydrochloride (7 mg/kg) was given intramuscularly as a sedative for transport to or from a standard restraining chair. All neurological assessments and physiological measurements were made at least 12 hours after sedation. Animals were conditioned in the restraining chair for several days prior to each experiment. A detailed description of the animal model has been published elsewhere and therefore only the salient features of the model will be presented here.

Under sodium pentobarbital (20 mg/kg), animals underwent implantation of intracerebral electrodes for monitoring of LCBF by hydrogen clearance. Five or more electrodes were implanted in cortical and subcortical structures ipsilateral to MCA occlusion, with a control electrode placed in the contralateral caudate nucleus. 5% hydrogen was administered via a plastic breathing chamber to saturation (about 20 minutes). Clearance curves for each electrode were recorded on a 12-channel Grass polygraph, and LCBF was calculated by single or double exponential analysis in cc/100 g/min. After placement of electrodes, awake animals had daily LCBF determinations (at least 3 each day) for control periods of 1–14 days. At the end of this period, animals were again anesthetized and underwent unilateral orbital exenteration and posterior orbital craniectomy for placement of a snare ligature about the origin of the MCA. One to 3 days following placement of the snare ligature device each awake animal was subjected to a period of temporary MCA occlusion of 15 minutes to 3 hours. LCBF was determined immediately before, during and after temporary occlusion. Post-occlusion LCBF measurements were made over 2 to 4 weeks. Neurological status was assessed during MCA occlusion and daily after de-occlusion until sacrifice. Neurological deficit was graded as follows: none; mild = barely detectable arm paresis or gaze preference to the side of occlusion; moderate = moderate hemiparesis, eye and head deviation toward the side of occlusion, +/− lethargy; and severe = virtual hemiplegia, with facial weakness, hemianopia, and lethargy.

Monkeys surviving MCA occlusion were sacrificed 2 weeks post-occlusion. Selected cases were allowed to survive 3 and 4 weeks. LCBF recording sites were marked in vivo, immediately prior to sacrifice, by applying a standardized monopolar cauterization current to each electrode. Brains were fixed in situ by transcardiac perfusion with 10% phosphate-buffered formalin. After removal, brains were placed in the same fixative for at least one week. The whole brain excluding the contents of the posterior fossa was then embedded in celloidin and serially sectioned in the horizontal plane. At intervals of 22 sections, three consecutive 15 μm sections were stained: one with hematoxylin and eosin, one with cresyl violet (Nissl), and one with the Loyez stain for myelin. Selected sections were stained with cresyl violet/Loyez and with the Gros/Bielschowsky stain for axons.

The presence of cerebral tissue damage and its precise relation to LCBF recording sites were determined by light microscopic examination of the tissue sections. The position of the electrode tip was identified and the surrounding local tissue reaction to the ischemic insult was assessed by one of us (U.DeG.) without knowledge of the LCBF data. The severity of local neuropathological damage observed in animals surviving the ischemic insult was graded on a three-point scale (fig. 1).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Neuropathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No lesion</td>
</tr>
<tr>
<td>B</td>
<td>Selective necrosis — incomplete tissue destruction</td>
</tr>
<tr>
<td>C</td>
<td>Total necrosis — complete tissue destruction</td>
</tr>
</tbody>
</table>

Results

Conditioning in the restraining chair appeared to calm monkeys during physiological measurements. Blood glucose levels were within normal limits in 5 animals tested on the day of MCA occlusion. Control LCBF data are summarized in table 1. Mean LCBF for separate brain regions was computed pooling determinations from many animals. Gray matter hydrogen clearance was bi-exponential in about a third of determinations, while white matter clearance was consistently mono-exponential. LCBF values tended to be both higher and more variable in gray matter than in white matter. LCBF values obtained from electrodes which were positioned at the border between gray and white matter are not included.

MCA Occlusion

Immediately after MCA occlusion, all animals developed some degree of neurologic deficit (see table 3). Gaze preference toward the side of occlusion appeared within minutes. In some monkeys, hemiparesis developed over a few minutes. After 30 minutes, deficits were maximal.

Decreases in ipsilateral LCBF were variable (table 2). The putamen underwent the greatest drop in flow, to about 33% of pre-occlusion levels, while other cortical and subcortical gray and white matter regions were reduced to about 50% of pre-occlusion LCBF levels. Clearance curves during MCA occlusion were always mono-exponential in the zone of flow reduction even when they had been bi-exponential throughout the pre-occlusion period. LCBF during occlusion at the site in
The neuropathologic evidence for tissue injury following MCA occlusion was generally unchanged from pre-occlusion (a 50% or greater increase at this site during occlusion occurred in 2 of 19 animals and a 50% or greater decrease occurred in 1 animal). Immediately upon release of MCA occlusion, LCBF in the ipsilateral hemisphere increased in every case in which it was decreased. Nine animals showed post-occlusion hyperemia (to 400% of pre-occlusion), which occurred at each site ipsilateral to occlusion to varying degrees and lasted less than 24 hours. An increase in contralateral LCBF occurred in 3 of 19 animals, but a substantial decrease in contralateral LCBF was never observed. The average LCBF, during the weeks following MCA occlusion, was generally unchanged from that prior to occlusion regardless of the duration of occlusion. Clinical data are summarized in table 3.

Neuropathology

The neuropathologic evidence for tissue injury following MCA occlusion in this model is variable in both topographic extent and severity.3,5,15,16 Table 3 gives the location and grade of the lesions found in relation to the duration of MCA occlusion and in association with the LCBF observed during occlusion. Lesions observed away from LCBF electrodes are indicated for each animal (table 3, region 'X'). Three animals, subjected to MCA occlusion of 15, 30 and 60 minutes duration, showed no cerebral tissue damage (grade A) by our histopathological analysis. Sixteen animals suffered tissue necrosis of varying severity (grade B and C) after occlusion of 1 to 3 hours. Three of these 16 animals, after occlusion for 1.5 and 2 hours, died at 24, 48 and 96 hours post-occlusion.

Total necrosis (grade C) was found in both gray and white matter. The light microscopic characteristics of total necrosis were (fig. 1): i) disappearance of all normal gray and white matter cellular elements, ii) partial liquefaction necrosis of devitalized tissue, iii) marked proliferation of phagocytic cells and thin walled blood vessels, particularly toward the center of the lesion, and iv) brisk reactive astrocytosis at the edges of the lesion.

Selective necrosis (grade B) was found only in the gray matter. This lesion was characterized by a distinctive pattern of cellular injury (fig. 1): i) disappearance of the cell bodies of neurons with relative preservation of the integrity of the tissue framework and of myelinated fibers in the injured region, ii) prominent microglial cell proliferation, and iii) moderate reactive astrocytosis. In animals that demonstrated selective necrosis the cellular reaction to injury evolved as the duration of survival was increased from 2 to 3 or 4 weeks. Older lesions tended to be somewhat more cellular. In decreasing order of susceptibility, the globus pallidus/putamen, caudate nucleus, claustrum, and insular cortex in the MCA territory of distribution were preferentially involved over other gray matter structures. Lesions in these gray matter areas were often multiple and stellate. The smallest lesions seemed to be around capillaries or small venules. A comparable lesion to selective gray matter necrosis was not found in white matter or in myelinated fibers in the gray matter. That is, when lesions were found in the white matter all components seemed to be uniformly destroyed.

There was some variability in the severity (selective vs. total necrosis), size and distribution of lesions in the gray matter of animals which underwent MCA occlusion for the same duration (see table 3). As the duration of MCA occlusion approached 2 hours, some animals evidenced areas of transition between selective and total necrosis.

Correlation of LCBF and Neuropathology

In Table 3, tissue status is correlated to LCBF during MCA occlusion. Data are presented graphically in figure 2. Absolute LCBF is plotted against duration of
TABLE 3  Summary of Clinical, LCBF and Neuropathological Data

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Duration of MCA occlusion</th>
<th>Survival</th>
<th>Neurological deficit</th>
<th>Electrode*</th>
<th>Region</th>
<th>LCBF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PATH&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>1</td>
<td>15 mins</td>
<td>4 wks</td>
<td>Moderate None</td>
<td>NS</td>
<td>6</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>30 mins</td>
<td>4 wks</td>
<td>Moderate None</td>
<td>NS</td>
<td>17</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 hr</td>
<td>3 wks</td>
<td>Mod-Sev None</td>
<td>NS</td>
<td>12</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 hr</td>
<td>3 wks</td>
<td>Moderate Mild</td>
<td>WM</td>
<td>7</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1 hr</td>
<td>4 wks</td>
<td>Moderate None</td>
<td>WM</td>
<td>11</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1 hr</td>
<td>2 wks</td>
<td>Moderate None</td>
<td>WM</td>
<td>20</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1 hr</td>
<td>2 wks</td>
<td>Moderate None</td>
<td>WM</td>
<td>16</td>
<td>A</td>
<td></td>
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<tr>
<td>8</td>
<td>1.25 hrs</td>
<td>2 wks</td>
<td>Moderate None</td>
<td>WM</td>
<td>11</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.5 hrs</td>
<td>4 days</td>
<td>Severe Death†</td>
<td>NS</td>
<td>4</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.5 hrs</td>
<td>2 wks</td>
<td>Moderate None</td>
<td>NS</td>
<td>10</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.75 hrs</td>
<td>2 wks</td>
<td>Mild-Mod Mild</td>
<td>NS</td>
<td>4</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2 hrs</td>
<td>2 wks</td>
<td>Moderate None</td>
<td>NS</td>
<td>9</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2 hrs</td>
<td>3 days</td>
<td>Severe Death†</td>
<td>WM</td>
<td>3</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>14</td>
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<td>1 day</td>
<td>Severe Death†</td>
<td>WM</td>
<td>5</td>
<td>A</td>
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<td>15</td>
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<td>3 wks</td>
<td>Moderate None</td>
<td>WM</td>
<td>17</td>
<td>A</td>
<td></td>
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</tbody>
</table>
occlusion, and tissue changes are indicated as none (grade A), selective necrosis (grade B), or total necrosis (grade C). Gray matter sites are graphed separately from white matter sites. Electrode tips positioned between gray and white matter or adjacent to damaged tissue are not included in these data. Critical levels of LCBF emerge as thresholds for necrosis. In the gray matter we found selective necrosis during ischemia (LCBF 5–15 cc/100 g/min) for 1–3 hours. When LCBF fell below 5 cc/100 g/min in the gray matter, total necrosis occurred. In the white matter, no tissue damage appeared until 2 hours, when LCBF below 5 cc/100 g/min was associated with total necrosis. In the gray matter, tissue damage occurs earlier and at a higher threshold of LCBF than in the white matter. When LCBF during occlusion was plotted as percent of pre-occlusion LCBF, the thresholds for tissue damage were substantially less clear in both gray and white matter.

Separate plots were made for neostriatum (caudate and putamen) and cortex (most often the insular cortex) (fig. 3). Selective necrosis was observed only in neostriatum. Though data are limited, total necrosis seems to occur at higher levels of LCBF in the neostriatum than in the cortex.

Post-occlusion LCBF did not correlate with the neuropathology. Acute post-occlusion hyperemia was observed in animals revealing total and selective necrosis as well as those showing no ischemic injury. Hemorrhage into tissue was not observed in animals with or without post-occlusion hyperemia.

Discussion

In comparison with other published values in the monkey and baboon these cortical LCBF values are lower, possibly related to hypocapnia, inhalation technique or our sampling from insular cortex. The variable reduction in LCBF following MCA occlusion may be attributed to variability in collateral supply.

The threshold concept, as discussed in detail elsewhere, is in evidence in figure 2. A critical level of gray matter flow of about 10–12 cc/100 g/min during 1–3 hours of MCA occlusion (fig. 2) is consistent with thresholds in previous studies. A gray matter CBF threshold “zone” (figure 2) includes LCBF values associated with no tissue injury as well as values associated with selective necrosis. This suggests differential vulnerability among individual gray matter sites. A gray matter CBF threshold for total necrosis only is lower than that described above. The shape of the developing thresholds, climbing steadily during the first 3 hours of ischemia, is evident and in agreement with our earlier work.

The concept of selective vulnerability in the nervous system is not new. It has been observed in human and experimental cerebro-vascular disease. In renal and cerebral ischemia, (both heterogeneous tissues), gradations of tissue destruction can be appreciated as selective necrosis of differentially susceptible elements. In relatively homogeneous tissues (heart, liver), the transition zone between normal and infarcted tissue is not always sharp and in this borderzone, partial or selective necrosis can also be seen (MacMao-
FIGURE 1. Examples of Selective and Total Necrosis as Result of MCA Occlusion (A) Insular isocortex, Normal {A} — Note regular lamination and even spacing of neurons and glia within each cortical layer, Cresyl violet, × 100. (B) Caudate nucleus, Normal {A} — Note uniform cellularity: small and large neurons and nuclei of glial cells. Bundles of myelinated fibers traverse the gray matter. Cresyl violet/Loyez, × 100. (C) Insular isocortex, Selective necrosis {B} — Note: 1) focal loss of neurons within several layers of the cortex, 2) microglial proliferation and reactive astrocytosis. Cresyl violet, × 100. (D) Caudate nucleus, selective necrosis {B} — Note: 1) patchy loss of neuronal cell bodies, 2) nests of reactive astrocytes in damaged areas, 3) preservation of myelinated fibers throughout. Cresyl violet/Loyez, × 100. (E) Putamen, Total necrosis {C} — Note central acellular region of liquefaction necrosis bounded by rim of hypercellularity composed of macrophages and reactive glia. Cresyl violet × 20. (F) Globus pallidus, Total necrosis {C} — Note cavitory infarction with destruction of all normal myelinated fibers within the infarcted area. Loyez, × 20.

According to Scholz\textsuperscript{25, 26, 27} selective necrosis in the CNS after an ischemic insult is related to: (1) the rapidity of the insult, (2) the severity and duration of ischemia, (3) the proximity of the lesion to other more severely damaged regions (i.e., edge of total infarct), (4) the differential sensitivity of constituent cells of the nervous system (neurons > oligodendrocytes > astro-
FIGURE 2. LCBF and Tissue Damage After MCA Occlusion. For individual electrodes, LCBF is plotted against duration of MCA occlusion. Each symbol represents one electrode and local tissue status is indicated by symbol shading. Data are presented separately for gray matter and white matter. Note that the CBF threshold for tissue damage is higher in the gray matter than in the white matter. Selective necrosis occurred only in gray matter.

FIGURE 3. LCBF and Gray Matter Damage After MCA Occlusion. Symbols and plotting as in figure 2 except electrodes from neostriatum and cortex are plotted separately. Data are limited but damage tends to appear earlier and at higher LCBF values in the neostriatum, suggesting possible greater vulnerability.
cytes > microglia) and (5) differences in vulnerability amongst various neurons. Several of these factors played a role in the present study. Certainly increasing duration and severity of ischemia led to increasing damage in every area studied. However, for equivalent duration and intensity of ischemia, gray matter showed consistently more severe histologic damage. Only differentially greater susceptibility of gray matter elements can explain these findings. This susceptibility may be related to higher metabolic requirements for maintenance of tissue integrity. The greater substrate demand in gray matter during rest, activation and anesthesia is consistent with this concept. Post-ischemic factors, such as uncoupling of flow and metabolism might also be important to regional vulnerability.

The relationship of blood flow to selective, incomplete or partial tissue injury has not been resolved in any tissue. However, our data indicate that it is the LCBF during occlusion and not the post-occlusion flow which correlates with the neuropathology. Two aspects of the results obtained in our study deserve emphasis: a) selective necrosis of gray matter was found when regional blood flow during occlusion dropped to an intermediate level between the lower range for total necrosis and the upper range of normal, and b) gradations of tissue injury were seen only in gray matter, not in white matter. This suggests that susceptible cells in gray matter (i.e., neurons) are more sensitive to equivalent reductions in blood flow than susceptible cells in white matter (i.e., oligodendrocytes).

The limited data in figure 3 suggest that subcortical gray matter is especially vulnerable to ischemia in comparison with the cortex. More experiments are needed to establish this point. Since these gray matter regions reveal similar rates of metabolism at rest, these data suggest that factors other than metabolic demand may play a role in their differential vulnerability.

References

Differential regional vulnerability in transient focal cerebral ischemia.
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