Correlation Between Cerebral Blood Flow and Histologic Changes in a New Rat Model of Middle Cerebral Artery Occlusion

Haruo Nagasawa, MD, and Kyuya Kogure, MD

We describe a new focal ischemia model consisting of unilateral middle cerebral artery occlusion with a silicone rubber cylinder attached to a nylon surgical thread inserted through the internal carotid artery in rats. Recirculation was accomplished by pulling the thread out of the artery. We evaluated the reliability of this model and studied the influence of reperfusion of the brain by measuring regional cerebral blood flow in 30 rats and by using conventional neuropathologic methods after different periods of occlusion in 48 rats. The anterior neocortex and the lateral part of the caudate putamen, which were supplied by the occluded middle cerebral artery, were the regions most frequently damaged. After 1 hour of occlusion in five rats, in the cortex supplied by the occluded artery mean±SD blood flow was 0.19±0.08 ml/g/min (approximately 15% of that in the corresponding region of five sham-operated control rats), and mild scattered ischemic cell change was observed. Three (n=5) or six (n=5) hours of occlusion reduced blood flow more severely and caused severe ischemic cell changes in the cortex supplied by the occluded artery in proportion to the duration of ischemia. Characteristically, in five rats subjected to 3 hours of occlusion followed by 3 hours of recirculation, blood flow was restored and spongy edematous change was observed in the cortex supplied by the recirculated artery. This change resulted in hypoperfusion of the neighboring cortical region surrounding the recirculated area. Our model should be useful in various investigations of the influence of reperfusion on focal ischemic brain injury. (Stroke 1989;20:1037-1043)

Recirculation affects cerebral ischemia and modifies postischemic events in various ways. Recirculation occurs frequently after spontaneous thrombolysis and break-up of cerebral emboli in a common clinical event. Focal ischemic models induced by occlusion of an intracranial artery, usually the proximal middle cerebral artery (MCA), have been widely studied in various animals such as cats, dogs, squirrel monkeys, and rats. It is difficult to produce reliable recirculation models using the above models because after recirculation cerebral blood flow (CBF) often varies regionally due to spasm or direct mechanical damage to the occluded vascular wall from clipping or ligation and because hemodynamic patterns are also variable due to changes in intracranial pressure caused by craniotomy. On the other hand, after an extracranial artery has been occluded without craniotomy, the development of ischemic brain damage can usually be prevented by an efficient intracranial collateral circulation unless additional stress (such as hypoxia or hypotension) is added or unless diffuse vascular disease is present. However, these models have many complicating factors due to such stresses. We have developed a new recirculation model in rats by occlusion of the MCA with no need for craniotomy. The reliability of our model has been evaluated by measuring CBF using an autoradiographic method and by conventional neuropathologic methods.

Materials and Methods

One hundred twenty-four adult male Wistar rats of the SPF strain weighing 280–300 g were allowed free access to food and water before and after all procedures.

After induction of anesthesia with a gas mixture of 70% N₂O and 2% halothane (the balance O₂), the rats were placed in the supine position. After a median incision of the neck skin, the right carotid artery was exposed with careful conservation of the vagus nerve. In 13 sham-operated control rats, the
right internal and external carotid arteries were ligated. In the remaining 111 rats, the right MCA was occluded with a silicone rubber cylinder attached to nylon surgical thread introduced from the bifurcation of the internal carotid artery immediately after ligation of the ipsilateral common and external carotid arteries. The cylinder was made of 4-0 nylon surgical thread (Nitto Kogyo Co., Ltd.) 16 mm long coated with silicone (Xantopren, Bayer Dental) mixed with a hardener (Elastomer Activator) to thicken the distal 5 mm to 0.25–0.30 mm in keeping with the method of Koizumi et al. The proximal tip of the thread was heated, creating a globular stopper for embolization and for easy removal of the cylinder (Figure 1). After introducing the embolus, the internal carotid artery was ligated just distal to the point of insertion. The embolus extended from the bifurcation of the internal carotid artery to the proximal portion of the anterior cerebral artery (ACA). The origin of the right MCA and posterior communicating artery was occluded by the silicone rubber cylinder (Figure 2A). The motor area of the frontoparietal cortex of the occluded side was supplied by the ipsilateral ACA via the anterior communicating artery from the contralateral internal carotid artery. The surgery was performed within 15 minutes with no bleeding. Body temperature was kept at normal limits with a heating pad.

Following surgery, anesthesia was discontinued and the rats were allowed free access to food and water until the next procedure was performed. All rats exhibited neurologic deficits characterized by left hemiparesis with upper extremity dominant and right Horner’s syndrome. Survival up to 7 days was assessed in 41 rats, and the ischemic area was confirmed by transcardiac perfusion with carbon black in five rats after 1 hour (1 rat), 3 hours (2 rats), and 6 hours (2 rats) of occlusion.

Recirculation was performed in 26 rats by pulling the thread out of the internal carotid artery (under the same anesthetic condition as surgery) after 1 hour of MCA occlusion in 13 rats and after 3 hours of MCA occlusion in the other 13 rats. In this model, the ischemic area could be reperfused via the cerebral arterial circle (circle of Willis) through the contralateral carotid artery, basilar artery, and collateral circulation of the cortical branches of the cerebral arteries since the ipsilateral common and external carotid arteries had been ligated (Figure 2B). Once again, the rats were allowed free access to food and water until the next procedure. Recirculation continued for 2 hours following 1 hour of MCA occlusion or for 3 hours following 3 hours of MCA occlusion. No subarachnoid hemorrhage or secondary cerebral embolism was noted in these 26 recirculated rats when the brains were removed (see below).

For the neuropathologic study, 48 rats were anesthetized following the procedures described above, and their brains were perfusion-fixed with 40% formaldehyde:glacial acetic acid:methanol (1:1:8; FAM) via the ascending aorta after briefly (30 seconds) washing out the cephalic circulation with heparinized physiological saline. The brains were removed from the skulls and stored in FAM until they were embedded in paraffin. Brain sections (5
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**Rat MCA Occlusion Model**

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FIGURE 3. Anatomic regions of coronal section through caudate putamen of rats for [14C]iodoantipyrine autoradiograms and to assess neuronal damage. 1 and 7, frontoparietal cortex, somatosensory area, supplied by middle cerebral artery; 2 and 6, lateral segment of caudate putamen; 3 and 5, medial segment of caudate putamen; 4, frontoparietal cortex, motor area, supplied by anterior cerebral artery. Shaded area represents ischemic area.

... were stained with cresyl violet and Luxol fast blue and with hematoxylin and eosin. The sections were examined under a light microscope, and regional ischemic neuronal damage was graded according to the number of cells with morphological changes and by the nature of the stainings. The regions studied included the anterior neocortex and caudate putamen, which were most frequently damaged in this ischemic model (Figure 3).

For the measurement of CBF in 30 rats, a tracheotomy was performed under the same anesthetic conditions and the rats were ventilated. Pancuronium bromide (0.6 mg/kg i.p.) was administered, and both femoral arteries and a femoral vein were cannulated. After surgical preparation, 2% halothane was discontinued and the rats were ventilated with 70% N2O and 30% O2, allowing normoxia and normocapnia. CBF was measured by the [14C]iodoantipyrine quantitative autoradiographic technique according to Sakurada et al. In brief, 20 µCi (0.6 ml) of 4-iodo-N-methyl-[14C]iodoantipyrine was infused intravenously over 30 seconds. During the infusion, several 20-µl samples of arterial blood from the free-flowing femoral artery catheter were collected in sample tubes. The [14C]iodoantipyrine concentration in the blood samples was determined by a liquid scintillation counter (Aloka) allowing 24 hours for decolorization in a mixture with 1 ml tissue and gel solubilizer (Protosol) and 100 µl H2O2. The rats were decapitated approximately 30 seconds after the start of infusion. The brains were quickly removed and frozen in powdered dry ice. Each brain was sectioned (20 µm) in a cryostat at −20°C, and the sections were exposed to x-ray film (Kodak NMC-1, Rochester, New York) with an autoradiographic carbon-14 standard microscale (Amersham) in x-ray cassettes for 2 weeks. Cerebral carbon-14 tissue concentrations were determined by means of a computer-based microdensitometer system (Chromoscan). CBF data were analyzed using a t test, with p<0.01 considered statistically significant.

**Results**

Figure 4 shows the extent of the ischemic area in this model demonstrated by transcardiac perfusion of five rats with carbon black after 1 hour, 3 hours,

![Diagram](https://example.com/diagram.png)

**TABLE 1. Distribution and Grade of Ischemic Neuronal Damage in Rats After Middle Cerebral Artery Occlusion**

<table>
<thead>
<tr>
<th>Region</th>
<th>Control</th>
<th>1 hr</th>
<th>3 hr</th>
<th>6 hr</th>
<th>1 hr+2 hr</th>
<th>3 hr+3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ipsilateral</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FrPaM</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>FrPaSS</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CPu(L)</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CPu(M)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Contralateral</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FrPaM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FrPaSS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CPu(L)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CPu(M)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are mean grade for eight rats by comparing with corresponding regions of sham-operated and control rats. 0, normal brain; 1, few neurons damaged; 2, many neurons damaged; 3, majority of neurons damaged. FrPaM, frontoparietal cortex, motor area, supplied by anterior cerebral artery; FrPaSS, frontoparietal cortex, somatosensory area, supplied by middle cerebral artery; CPu(L), lateral segment of caudate putamen; CPu(M), medial segment of caudate putamen.
TABLE 2. Physiological Variables in Measurement of Cerebral Blood Flow in Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Occlusion 1 hr</th>
<th>Occlusion 3 hr</th>
<th>Occlusion 6 hr</th>
<th>Occlusion+recirculation 1 hr+2 hr</th>
<th>Occlusion+recirculation 3 hr+3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>137±9</td>
<td>137±9</td>
<td>123±20</td>
<td>120±20</td>
<td>133±23</td>
<td>132±24</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.8±0.2</td>
<td>37.0±0.4</td>
<td>36.9±0.1</td>
<td>36.6±0.3</td>
<td>36.7±0.4</td>
<td>36.4±0.4</td>
</tr>
<tr>
<td>Pao2 (mm Hg)</td>
<td>110±11</td>
<td>128±2</td>
<td>127±8</td>
<td>145±4</td>
<td>118±16</td>
<td>139±3</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>38.9±1.1</td>
<td>40.4±2.9</td>
<td>39.1±2.3</td>
<td>39.2±0.9</td>
<td>40.0±1.7</td>
<td>41.1±1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.34±0.14</td>
<td>7.41±0.07</td>
<td>7.50±0.07</td>
<td>7.39±0.13</td>
<td>7.50±0.03</td>
<td>7.49±0.02</td>
</tr>
</tbody>
</table>

Data are mean±SD for five rats.

Table 2 shows the mean arterial blood pressure, body temperature, and blood gas tension (Pao2, Paco2, and pH) of the sham-operated control and occluded rats. There were no significant differences between groups. In the control rats, mean±SD CBF in the cortex was 1.46±0.29 ml/g/min. One hour of MCA occlusion reduced CBF to 0.19±0.08 ml/g/min, followed by 1 hour of MCA occlusion with no recirculation. Irreversible necrotic changes in brains after 3 hours of MCA occlusion were observed in the frontal-parietal cortex and lateral parts of the caudate nucleus and thalamus. The grade of ischemic damage in rat brains after 3 or 6 hours of MCA occlusion was proportional to the duration of the occlusion. There was also evidence of mild ischemic changes in neurons of the frontal-parietal cortex in the ipsilateral ACA area, proportional to some extent to the duration of the occlusion. Mild ischemic changes in the brains of rats subjected to 3 hours of MCA occlusion followed by 3 hours of recirculation were observed not only in the frontal-parietal cortex of the ipsilateral ACA territory, but also in the neocortex of the contralateral hemisphere. However, evidence of ischemic changes in the contralateral hemisphere was not significant compared with that of rats subjected to 6 hours of MCA occlusion. No histologic abnormalities were seen in the cerebellum or brainstem.

TABLE 3. Regional Cerebral Blood Flow in Rats After Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Region</th>
<th>Control</th>
<th>Occlusion 1 hr</th>
<th>Occlusion 3 hr</th>
<th>Occlusion 6 hr</th>
<th>Occlusion+recirculation 1 hr+2 hr</th>
<th>Occlusion+recirculation 3 hr+3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FrPaM</td>
<td>1.58±0.25</td>
<td>0.94±0.10*</td>
<td>0.88±0.04*</td>
<td>0.75±0.19*</td>
<td>1.78±0.22</td>
<td>0.56±0.03†</td>
</tr>
<tr>
<td>FrPaSS</td>
<td>1.42±0.21</td>
<td>0.19±0.08*</td>
<td>0.09±0.04*</td>
<td>0.08±0.06*</td>
<td>0.96±0.22†</td>
<td>1.56±0.18†</td>
</tr>
<tr>
<td>CPu(L)</td>
<td>1.62±0.19</td>
<td>0.04±0.02*</td>
<td>0.03±0.02*</td>
<td>0.02±0.01*</td>
<td>0.97±0.18†</td>
<td>1.33±0.15‡</td>
</tr>
<tr>
<td>CPu(M)</td>
<td>1.53±0.14</td>
<td>0.62±0.15*</td>
<td>0.53±0.10*</td>
<td>0.49±0.14*</td>
<td>1.35±0.10</td>
<td>0.77±0.12†</td>
</tr>
<tr>
<td>Contralateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FrPaM</td>
<td>1.54±0.22</td>
<td>1.49±0.15</td>
<td>1.43±0.23</td>
<td>1.52±0.25</td>
<td>1.83±0.19</td>
<td>1.44±0.15</td>
</tr>
<tr>
<td>FrPaSS</td>
<td>1.39±0.19</td>
<td>1.69±0.16</td>
<td>1.59±0.22</td>
<td>1.17±0.20</td>
<td>1.88±0.17</td>
<td>1.42±0.18</td>
</tr>
<tr>
<td>CPu(L)</td>
<td>1.65±0.17</td>
<td>1.63±0.12</td>
<td>1.65±0.17</td>
<td>1.31±0.23</td>
<td>1.77±0.22</td>
<td>1.41±0.21</td>
</tr>
<tr>
<td>CPu(M)</td>
<td>1.55±0.12</td>
<td>1.73±0.13</td>
<td>1.60±0.18</td>
<td>1.42±0.16</td>
<td>1.76±0.13</td>
<td>1.46±0.19</td>
</tr>
</tbody>
</table>

Data are mean±SD ml/g/min for five rats. FrPaM, frontoparietal cortex, motor area, supplied by anterior cerebral artery; FrPaSS, frontoparietal cortex, somatosensory area, supplied by middle cerebral artery; CPu(L), lateral segment of caudate putamen; CPu(M), medial segment of caudate putamen.

*p<0.01 different from control.
†p<0.01 different from control and other occlusion+recirculation value.
ml/g/min in the cortex supplied by the occluded MCA (approximately 15% of that in corresponding regions of the control rats). CBF was further reduced in proportion to the duration of the occlusion (Table 3); 3 and 6 hours of MCA occlusion reduced CBF to 0.09±0.04 and 0.08±0.06 ml/g/min in the cortex supplied by the occluded MCA, respectively (approximately 6% of that in corresponding regions of the control rats). In the cortex supplied by the ipsilateral ACA, CBF ranged from 0.94±0.10 to 0.75±0.19 ml/g/min, depending on the duration of the occlusion. In the cortex supplied by the contralateral MCA, CBF was not different from that of control rats. After 1 hour of MCA occlusion followed by 2 hours of recirculation, CBF in the cortex supplied by the recirculated MCA was restored to approximately 70% of the control value. After 3 hours of occlusion followed by 3 hours of recirculation, CBF in the cortex supplied by the ipsilateral ACA was 0.56±0.03 ml/g/min (Table 3), roughly 35% of that in the corresponding regions of the control rats and significantly different from that in the control rats, in rats subjected to 1 hour of MCA occlusion followed by 2 hours of recirculation, and in rats subjected to 3 hours of MCA occlusion only (Table 3).
Discussion

The reversibility of ischemic brain damage depends on the duration as well as on the severity of ischemia. Our reversible embolic method resulted in either a permanently or a transiently ischemic region in the MCA territory with no surgical manipulation of the cranial vault. In this ischemic model, the lateral segment of the caudate nucleus was most frequently damaged, and CBF in this ischemic core was reduced severely in the occluded hemisphere. A well-defined CBF threshold (0.12–0.15 ml/g/min for 2 hours) is of critical importance in the development of irreversible ischemic changes. In the cortex, 1 hour of MCA occlusion did not reduce CBF below the threshold and did not cause irreversible ischemic injury. Ischemic change was milder in the same cortical area after 1 hour of MCA occlusion followed by 2 hours of recirculation; in these rats, recirculation assisted recovery. The histologic criteria of irreversible cerebral damage during acute ischemia remain controversial. Long-term observation after 1 hour of MCA occlusion followed by recirculation is necessary to determine whether delayed neuronal damage occurs. Delayed neuronal necrosis has been reported in the CA1 sector of the hippocampus after transient ischemia in another global ischemia model.

After 3 or 6 hours of MCA occlusion, light microscopy revealed typical ischemic and dark cell changes, with most neurons shrunken and triangular with dark-stained nuclei, only within regions of the brain supplied by the occluded MCA; such severe damage suggested irreversible change, that is, cerebral infarction. Also, according to the [14C]iodoantipyrine autoradiograms, the ischemic area extended from the ischemic core to the surrounding area, with varying degrees of damage depending on the duration of MCA occlusion (Figure 5). After 6 hours of MCA occlusion, CBF was reduced slightly in the cortex supplied by the contralateral MCA. Light microscopy revealed mild ischemic changes, with some neurons shrunken and triangular with dark-stained nuclei, only in the cortex supplied by the contralateral MCA. Hypoperfusion in the contralateral cortex may be attributed to interhemispheric diascisis during prolonged ischemia.

In previous studies, reperfusion frequently resulted in inhomogenous blood flow in the reperfused areas. In our model, however, inhomogeneous blood flow was not observed in rats in which recirculation followed MCA occlusion. We demonstrated that recirculation could be performed completely since there was no direct damage to the occluded vascular wall during the preceding ischemia. After 3 hours of MCA occlusion followed by 3 hours of recirculation, CBF in the ipsilateral cortex was similar to that in the contralateral cortex and there was no significant difference between CBF in the recirculated area and the corresponding area of the control rats. Normal or rather high CBF in dying brain tissue is commonly seen, for example, in the CA1 sector of the hippocampus during delayed necrosis, presumably due to tissue acidosis. However, we were surprised to find that normal tissue perfusion can be maintained even in areas with marked edematous spongiform change and in necrotic brain mass. An explanation of this phenomenon is not available at present. After 3 hours of MCA occlusion followed by 3 hours of recirculation, CBF in the ipsilateral ACA territory was significantly reduced compared with that in both MCA territories (p<0.01). In these rats, marked focal brain edema caused by reperfusion of the ischemic area seemed to reduce CBF in the area just outside the border of the recirculated area.

The advantage of our model is that focal ischemic brain damage can be induced by a simple technique without craniotomy, which makes it possible to study the pathophysiologic mechanisms of brain damage during and after ischemia. Our novel ischemic model may be helpful in various investigations of the influence of reperfusion on focal ischemic brain injury.

References


KEY WORDS • animal models • cerebral blood flow • histology • rats
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Stroke. 1989;20:1037-1043
doi: 10.1161/01.STR.20.8.1037

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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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