Rat Middle Cerebral Artery Occlusion: Evaluation of the Model and Development of a Neurologic Examination

Joshua B. Bederson, M.D.*, Lawrence H. Pitts, M.D.*, Miles Tsuji, B.S.*, Merry C. Nishimura, B.S.*, Richard L. Davis, M.D.,† and Henry Bartkowski, M.D., Ph.D.*

SUMMARY We have examined the incidence and size of infarction after occlusion of different portions of the rat middle cerebral artery (MCA) in order to define the reliability and predictability of this model of brain ischemia. We developed a neurologic examination and have correlated changes in neurologic status with the size and location of areas of infarction.

The MCA was surgically occluded at different sites in six groups of normal rats. After 24 hr, rats were evaluated for the extent of neurologic deficits and graded as having severe, moderate, or no deficit using a new examination developed for this model. After rats were sacrificed, the incidence of infarction was determined at histologic examination. In a subset of rats, the size of the area of infarction was measured as a percent of the area of a standard coronal section.

Focal (1–2 mm) occlusion of the MCA at its origin, at the olfactory tract, or lateral to the inferior cerebral vein produced infarction in 13%, 67%, and 0% of rats, respectively (N = 38) and produced variable neurologic deficits. However, more extensive (3 or 6 mm) occlusion of the MCA beginning proximal to the olfactory tract — thus isolating lenticulostriate end-arteries from the proximal and distal supply — produced infarctions of uniform size, location, and with severe neurologic deficit (Grade 2) in 100% of rats (N = 17). Neurologic deficit correlated significantly with the size of the infarcted area (Grade 2, N = 17, 28 ± 5% infarction; Grade 1, N = 5, 19 ± 5%; Grade 0, N = 3, 10 ± 2%; p < 0.05).

We have characterized precise anatomic sites of the MCA that when surgically occluded reliably produce uniform cerebral infarction in rats, and have developed a neurologic grading system that can be used to evaluate the effects of cerebral ischemia rapidly and accurately. The model will be useful for experimental assessment of new therapies for irreversible cerebral ischemia.

DEVELOPMENT of a reproducible, reliable animal model of cerebral ischemia would allow the study of the pathophysiology of the lesion and the efficacy of various treatment modalities. Characteristics of models are based on similarities with syndromes of human cerebrovascular disease; a review of available models of focal ischemic infarction has been published recently. Ideally, experimental occlusion with or without reperfusion is accompanied by predictable changes in blood flow and a consistent degree of infarction that produces lesions of predictable location and size. To insure that the neurologic examination is reliable, animals should be neurologically similar to humans in terms of behavior, sensory-motor integration, and relative amount of neocortex. These requirements are best met by occlusion of a single artery in subhuman primates. However, primates are costly and difficult to maintain and therefore cannot be used by the majority of investigators who study cerebral ischemia.

The laboratory rat is a well-studied, relatively inexpensive, and readily available animal that has been used widely for fundamental studies of metabolism, neurochemistry, and neurophysiology. Rat models of cerebral ischemia are numerous, including bilateral carotid occlusion, intracranial compression that produces ischemia and increased intracranial pressure, (unilateral) carotid occlusion plus hypoxia or hypotension, compression of the neck with a cuff, arterial microembolization, and four vessel occlusion. While these methods have been useful to examine various aspects of cerebral ischemia, they have not provided a reliable model of focal cerebral ischemia.

Occlusion of the middle cerebral artery (MCA) of the rat has been used since 1975. This technique has been refined and has been used for several recent studies of focal cerebral ischemia. Our early experience with this model suggested that a 100% incidence of infarction was not produced by focal occlusion of the MCA even if occlusion was performed proximal to the olfactory tract, as has been reported. Although neurological abnormalities that occur after cortical ablation in the rat have been documented, a simple, reliable method with which to assess the severity of neurological deficits after the occurrence of stroke in rats has not been available. Because of these limitations the rat model has not been used widely to study the effects of cerebral ischemia.

Therefore, we have investigated the effect on the production of uniform site and size of areas of infarction caused by occlusion of the MCA at various sites, and have developed a simple, reliable method for assessment of neurological status of rats after occlusion of the MCA.
Methods

Seventy young adult male Sprague Dawley rats weighing 300-400 gm were allowed free access to food and water before and after all procedures. Rats were weighed and placed in an ether jar until they were immobilized, and anesthetized with 3.5% chloral hydrate in normal saline (35 mg/100 gm, intraperitoneally), which was supplemented as necessary during the procedure. Body temperature was monitored and maintained within normal limits with a heating pad.

Under the operating microscope the left MCA was exposed transcranially20 without damage to the zygomatic bone. Transection of the facial nerve was avoided during exposure of the temporalis muscle, which was divided caudally and retracted inferiorly to avoid compression of the orbital contents. The circle of Willis and the origin of the MCA was exposed in all rats by gently retracting the brain with a spatula on a flexible arm. The MCA was occluded with microbipolar coagulation using a low power setting and continuous saline irrigation, and then transected to avoid recanalization. Temporalis muscle and skin were closed in layers, and rats were allowed to recover from anesthesia on the heating pad. They were returned to their cages for the remainder of the 24-hour period.

Rats were randomized into six groups (fig. 1): Group 1, occlusion of the MCA from its origin to its junction with the inferior cerebral vein (N = 9); Group 2, occlusion from 2 mm proximal to the olfactory tract to the inferior cerebral vein (N = 10); Group 3, focal occlusion just proximal to the olfactory tract (N = 12); Group 4, focal occlusion at the origin of the MCA from the internal carotid artery (N = 15); Group 5, focal occlusion of the MCA beginning 1 mm distal to the inferior cerebral vein (N = 11). Brains were retracted for 5 to 7 min in Groups 1 and 4, 2 min in Groups 2 and 3, and 1 min in Group 5. Brain retraction alone was performed in Group 6 for 15 (a, N = 5), 20 (b, N = 5), or 25 (c, N = 3) min. In experienced hands, the procedure could be performed in 10-20 min (Groups 2, 3, 5), 15-25 min (Group 6), and 20-30 min (Groups 1, 4).

The neurologic status of each rat was evaluated carefully 24 hr after surgery by an observer who had no knowledge of which procedure had been performed. A grading scale of 0-3 was used to assess the effects of occlusion (table 1). The tests described below were conducted sequentially; if a rat exhibited the appropriate behavior at one step but not at the subsequent step, it was graded as the former.

Rats were held gently by the tail, suspended one meter above the floor, and observed for forelimb flexion. Normal rats extend both forelimbs toward the floor. Rats that extended both forelimbs toward the floor and that had no other neurological deficit were assigned grade 0. Rats with infarction consistently flexed the forelimb contralateral to the injured hemisphere; posture varied from mild wrist flexion and shoulder adduction with extension at the elbow to severe posturing with full flexion of wrist, elbow, and adduction with internal rotation of the shoulder. Rats with any amount of consistent forelimb flexion and no other abnormality were graded 1. Rats were placed on a large sheet of soft, plastic coated paper (counter protection paper, Kimberly Clarke) that could be gripped firmly by their claws. With the tail held by hand, gentle lateral pressure was applied behind the rat’s shoulder until the forelimbs slid several inches. The maneuver was repeated several times in each direction. Normal or mildly dysfunctional rats resisted sliding equally in both directions. Severely dysfunctional rats had consistently reduced resistance to lateral push toward the paretic side, and were graded 2. Rats were then allowed to move about freely and were observed for circling behavior. Rats that circled toward the paretic side consistently were graded 3. Forelimb flexion was always observed in rats with decreased resistance to lateral push; both forelimb flexion and decreased resistance to lateral push were always observed in rats that displayed circling behavior. The neurologic examination can be performed in 3 to 5 min.

| Table 1  Neurologic Examination Grading System |
|-------------------|-------------------|
| Normal            | grade 0: no observable deficit |
| Moderate          | grade 1: forelimb flexion |
| Severe            | grade 2: decreased resistance to lateral push (and forelimb flexion) without circling |
|                   | grade 3: same behavior as grade 2, with circling |

FIGURE 1. Locations of the middle cerebral artery occlusion and the infarction rates for the six groups. Infarction rate is the percent of rats that were surgically occluded that developed infarctions.
Immediately after the examination, rats were immobilized with ether and sacrificed with an intracardiac injection of sodium pentobarbital. Brains were removed rapidly and within 3 min of death coronal slices were made at 5 and 7 mm from the frontal tips, and sections were immersed in 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 37°C for 30 min; the presence or absence of infarction was determined in all rats by examining TTC-stained sections for areas on the side of infarction that did not stain with TTC (J. H. Bederson, L. H. Pitts, M. C. Nishimura, R. L. Davis, and H. M. Bartkowski, Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for the detection and quantification of experimental cerebral infarction in rats, revision submitted for publication). Sections were then transferred to 10% phosphate buffered formalin for fixation.

The size of infarction was calculated for 25 rats with different neurologic grades using the following method: 1 to 5 days after fixation, the rostral surface of the 2 mm thick TTC-stained section was photographed using color slide film (Ektachrome, Tungsten ASA 160). Histologic sections stained with hematoxylin and eosin (H & E) were then prepared from the same surface of this slice and were reviewed by a neuropathologist who had no knowledge of the experimental group to which the rats belonged.

Tracings of projected TTC and H & E slides were made by an observer unaware of the site of occlusion or the neurologic grade of the rat. The cortex and basal ganglia were outlined separately and the infarcted area was quantified by computerized image analysis systems and by cutting and weighing traced sections of normal and infarcted areas. The area of infarction in cortex and basal ganglia was expressed as a percent of the whole coronal section. Data were analyzed for correlations between neurological grade and infarction size.

Results

There were no operative deaths and seizures were not observed in rats after surgery.

The incidence of infarction after occlusion of the MCA was calculated as the percentage of rats in each surgical group that had identifiable areas of infarction on examination of TTC-stained brain slices (fig. 1). Rats in Group 1 (extensive occlusion) had an incidence of infarction of 100% (N = 9) and uniformly "severe" neurologic deficits (Grade 2, 100%). Rats in Group 2 (N = 10) had a 100% incidence and neurologic grades of 0 (1 rat), 1 (1 rat), or 2 (8 rats). Rats in Group 3 (N = 12) had a 67% incidence and neurologic grades of 0 (4 rats), 1 (5 rats), or 2 (3 rats). Infarction in Group 3 rats was located primarily in the basal ganglia, with relatively small areas of cortex affected. Rats in Group 4 (N = 15) had an incidence of infarction of only 13% with neurologic grades of 0 (13 rats), 1 (1 rat), and 2 (1 rat).

Rats in Groups 6a and b (brain retraction alone for 20 min or less) and Group 5 (distal occlusion of the MCA) had no infarction and a normal neurological exam. One of the three rats that had brain retraction performed for 25 min (Group 6c) had a small area of infarction in the medial basal ganglia that caused no neurological deficit.

Areas of infarction were determined by examination of both TTC and H & E stained sections. In 25 rats with histologically-documented infarction, the mean size of the area of infarction of basal ganglia and cortex (as a percentage of the area of the coronal section) was determined and compared with neurologic grades (table 2, fig. 2). The location of averaged areas of infarction for each neurologic grade are shown in figure 3. Of these 25 rats, 88% had a neurologic deficit, a sensitivity of 88% for the detection of histologic infarction using the neurologic assessment scale. All rats with areas of infarction greater than 20% of the coronal section had neurologic deficit, and all rats with neurologic deficit had histologically-documented infarction (no false positives).

The mean size of areas of infarction for Grade 2 and 3 rats was not significantly different (fig. 2), which indicated that the reduced ability to resist lateral push with or without circling behavior was predictive of large areas of infarction. Therefore, results for Grade 2 and 3 rats were combined. The size of the infarcted area for the combined "severe" group was 28 ± 5% (mean ± S.E.), which was significantly greater than the size of infarction for the "normal" Grade 0 rats (10 ± 2%, p < 0.01) or "moderate" Grade 1 rats (19 ± 5%, p < 0.025, table 2). The size of the infarcted area was significantly greater for Grade 1 than for Grade 0 rats (p < 0.05).

Discussion

A major limitation of the rat model of cerebral ischemia has been the lack of an accurate method with which to assess neurological abnormalities after cerebral infarction has been produced. 2, 29 Motor disturbances including hemiparesis are well known sequelae of lesions of frontal cortex and striatum in the rat, and the severity of neurological deficit has been correlated with an increase in the size of the lesion. 25, 26, 30 Neurologic deficits in rats are detected more consistently when specific tests rather than observation of spontaneous activity are used, 27 and subtle deficits can be detected with more rigorous tests. 22 In addition to motor abnormalities, a variety of behavioral and learning

Table 2 Percent Size of Infarction Versus Neurologic Outcome (grade)

<table>
<thead>
<tr>
<th>Area</th>
<th>Normal (0)</th>
<th>Moderate (1)</th>
<th>Severe (2)</th>
<th>2 + 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal ganglia plus cortex</td>
<td>10 ± 2</td>
<td>19 ± 5*</td>
<td>27 ± 6</td>
<td>27 ± 8</td>
</tr>
<tr>
<td></td>
<td>4 ± 2</td>
<td>11 ± 7</td>
<td>16 ± 8</td>
<td>15 ± 6</td>
</tr>
<tr>
<td></td>
<td>6 ± 4</td>
<td>8 ± 5</td>
<td>11 ± 2</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>5</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

*p < 0.05 ("moderate" vs "normal"),

‡p < 0.025 ("severe" vs "moderate").
A RAT MODEL FOR CEREBRAL INFARCTION Bederson et al

Grade 2
Grade 1
Grade 0

FIGURE 2. Infarction size (percent of coronal section) versus neurologic grade.

patterns of rats are affected by brain lesions, which may be the result of altered norepinephrine levels rather than of destruction of specific motor or sensory pathways.

We have developed a rat neurological examination that can be used to distinguish, with respect to the size of the area of infarction, between groups of rats in which the MCA was occluded. Advantages of this examination are its simplicity, 100% specificity, and the ability to distinguish between rats with small, moderate, and large areas of infarction.

The lateral portion of the anterior basal ganglia in rats is supplied by lenticulostriate branches of the MCA arising proximal to the olfactory tract, medially from Heubner's artery that arises from the anterior cerebral artery, and posteriorly from the medial lenticulostriate branches of the MCA. Therefore, occlusion of the MCA lateral to the olfactory tract is lateral to basal ganglia arterial supply, and occlusion of the MCA at its origin requires that the lateral basal ganglia be supplied by collateral flow if it is to remain viable.

While the vascular anatomy of rodents and primates is similar, during neonatal life in rodents the abundant collaterals between distal branches of the anterior, middle, and posterior cerebral arteries do not undergo considerable regression as they do in humans and other primates. Thus, occlusion of the MCA at its origin in primates severely reduces flow in basal ganglia and moderately reduces flow in the cortex and leads to a variety of histologic changes in the two areas.

The incidence of infarction in Groups 1 and 2 (extensive occlusion of the MCA) was 100%, while the incidence in Group 4 rats (focal occlusion of the MCA at its origin) was only 13%. The low incidence of infarction in Group 4 rats was probably the result of the persistence of abundant collateral circulation in rodents. Focal occlusion of the MCA just proximal to the olfactory tract (Group 3) produced a 67% incidence of infarction. Taken together, these findings indicate that isolation of lenticulostriate and small cortical arteries from both proximal and distal collateral supplies is necessary to produce infarction in 100% of rats. The difference between the incidence of infarction that we observed in Group 3 rats and that reported by others may be the result of the extremely focal nature of our occlusion technique, which may spare lenticulostriate and small cortical arteries despite its proximal location.

Occlusion of the MCA starting either at its origin (Group 1) or 2 mm proximal to the olfactory tract (Group 2) and extending to the inferior cerebral vein resulted in an incidence of infarction of 100%. Thus, more extensive occlusion produced lesions of predictable location and extent and was associated with consistent neurologic deficits. While more extensive surgery and brain retraction were necessary, this technique may be the most reliable of the rat MCA occlusion techniques reported.

The model of focal cerebral ischemic infarction reported here has both advantages and disadvantages compared with the single artery occlusion in subhuman primates. Exposure of the brain to air during craniectomy may alter intracranial pressure and blood-brain barrier permeability after infarction has been produced. However, in our studies retraction of the brain for less than 20 min did not produce any histologically-identifiable abnormality; even so, retraction may transiently alter blood-brain barrier permeability. The use of bipolar coagulation and the extensive occlusion necessary to reliably produce infarction hinders the study of collateral flow and reperfusion phenomena, which are important for the investigation of transient ischemia. Temporary occlusion of the internal carotid artery at the circle of Willis either directly by microclipping or indirectly by microcatheter techniques, which would reduce collateral flow to the MCA by limiting proximal supply to the anterior and
posterior cerebral arteries, could be used to avoid the latter problems. Barbiturates and general anesthesia may alter outcome in studies of cerebral infarction\(^{11,41}\) and prevent the immediate assessment of neurological changes after occlusion of the MCA.

Major advantages of the model reported here are a 100% incidence of infarction (in Groups 1 and 2), the predictable location and size of area of infarction, the consistent production of neurological deficits, and the availability and low cost of rats. Because the model is reproducible, the effects of various therapeutic agents on neurological outcome and size of infarction produced by focal cerebral ischemia can be studied.\(^{21}\)

Our technique for the assessment of neurologic function currently is limited by the 88.0% sensitivity. Three rats with areas of infarction of 9.9 ± 0.2% that were confined to small areas of the caudate-putamen and dorsolateral cortex (fig. 3) were graded 0. Ablation of dorsolateral frontal cortex has no effect on circling behavior or performance in a Y maze for rats,\(^{27}\) which confirms that lesions in this location do not produce deficits that can be assessed readily. A more rigorous motor task such as one requiring fine digital manipulation\(^{22}\) might detect the missed abnormalities.

\[\text{References}\]

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