Middle Cerebral Artery Occlusion Without Craniectomy in Rats
Which Method Works Best?

Rodney J. Laing, FRCS; Jan Jakubowski, MD, FRCS; and Robert W. Laing, MB, CHB

Background and Purpose: Our purpose was to assess the effectiveness of middle cerebral artery occlusion in producing acute focal ischemia in the rat by the use of Koizumi’s and Longa’s methods, in which occlusion is achieved by passing a nylon thread into the internal carotid artery.

Methods: Cerebral blood flow was measured by using the hydrogen clearance method, and the brains were examined histologically to assess ischemic damage.

Results: By Koizumi’s method profound reduction in cerebral blood flow was achieved in 28 of 30 rats (93%). The mean cerebral blood flow in the middle cerebral artery territory was 10.7 (95% confidence interval, 9.9–11.5) ml/100 g per minute. By Longa’s method reduction in cerebral blood flow was achieved in only 29 of 52 rats (56%), and in these animals mean cerebral blood flow was 33 (95% confidence interval, 28.3–33.7) ml/100 g per minute (p<0.001 compared with Koizumi’s method). Cerebral blood flow was reduced to <16 ml/100 g per minute in only seven animals (24%).

Conclusions: By Koizumi’s method the depth of ischemia is more profound, occlusion is achieved in a much higher proportion of cases, and the incidence of perforation of the intracranial internal carotid is much less frequent than by Longa’s method. (Stroke 1993;24:294–298)

Key Words • animal models • cerebral blood flow • cerebral ischemia • rats

For many years investigators have sought a minimally invasive rodent model of focal ischemia.

The main advantages of rodent models are low cost, close similarity of the cerebrovascular anatomy and physiology to higher species, and small brain size that is well suited to fixation procedures. For a model of focal ischemia to be useful in the experimental context it must be capable of producing an adequate level of ischemia as well as consistent and reproducible results. Branston’s group1 has established that neuronal function will fail when cerebral blood flow (CBF) falls to <16 ml/100 g per minute and that metabolic failure occurs when CBF is reduced to <12 ml/100 g per minute. In addition, the technique should be simple and have low mortality.

Models of focal ischemia in the rat have normally been produced by occlusion of the middle cerebral artery (MCA). The various models have recently been summarized.2 Most involve craniectomy with diathermy or ligature occlusion of the proximal MCA. More recently photochemical occlusion by the use of rose bengal and laser has been described,3 and in some models the MCA occlusion has been supplemented with extracranial vascular occlusion or induced hypotension.4

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These techniques are invasive and of varying degrees of complexity, and in most it is not possible to achieve reperfusion. In 1986 Koizumi et al5 published a novel method of reversible MCA occlusion that used an intravascular embolus. This model was further evaluated by Nagasawa and Kogure,6 who in 1989 correlated histological changes in the vasculature with CBF. In 1989 Longa’s group7 published a variation on this method and stated that their technique reliably produced regional infarcts in rats weighing 300–400 g. We adopted Longa’s method to study focal ischemia in the rat but found that the technique produced inconsistent results. We then adopted Koizumi’s method and found the technique reliable and effective. This article compares the effectiveness and reliability of these methods in the production of acute ischemia in the MCA territory.

Materials and Methods

One hundred adult Wistar rats weighing 300–400 g were anesthetized with ether and an intraperitoneal injection of Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml) mixed with midazolam 5 mg/ml and diluted with water. A tracheostomy was performed, and the rat was ventilated with a mixture of oxygen and air. During ventilation the rat was paralyzed with gallamine, and anesthesia was maintained with repeated intraperitoneal injections of Hypnorm and midazolam as necessary. The femoral artery was cannulated for blood pressure monitoring, blood sampling, and fluid administration. To ensure maintenance of a steady-state
blood pressure, blood gases and temperature were monitored and maintained within narrow limits.

Two burr holes were made on the lateral aspect of the skull on each side, and 2-mm-long platinum electrodes were placed through the dura into the brain, one positioned to lie in the center of the MCA territory and one on the posterior margin of this territory. CBF was measured by the hydrogen clearance technique, in which the current of hydrogen ionization is detected by platinum electrodes, digitalized by computer, and displayed in a graphic form on a screen. In this way the adequacy of MCA occlusion could be assessed dynamically and compared with control flows in the opposite hemisphere.

In 18 control rats CBF and physiological variables were measured repeatedly for up to 7 hours to establish the stability of the preparation. Occlusion was attempted in 82 rats.

The two methods of producing MCA occlusion both involve the introduction of an intraluminal 4-0 surgical thread to occlude the origin of the MCA. The carotid bifurcation in the neck is exposed, the common carotid artery is occluded, and the branches of the external carotid artery are dissected and divided. The internal carotid artery is followed rostrally, and the pterygopalatine branch is identified and divided. The thread is then introduced into the internal carotid artery and advanced between 17 and 20 mm.

The difference in the techniques described by Koizumi and Longa lies in the preparation of the thread. In Longa’s method the tip of the suture is blunted by heating near a flame, and in Koizumi’s method the distal 5 mm is coated with silicone to a diameter of 0.25 mm (see References 5 and 7 for details). Both types of thread are easily and reproducibly made (Figure 1).

To help with correct positioning of the suture a hydrogen desaturation curve was recorded during its introduction, and an abrupt change in the slope of the curve indicated successful occlusion of the MCA (Figure 2). After induction of ischemia, CBF was measured repeatedly for 4 hours, and then the animal was killed. The thread was withdrawn, and the rat was decapitated. The brain with the vessels constituting the circle of Willis attached was removed intact and examined with an operating microscope to confirm the positioning of the thread. It was quite clear exactly where the suture had been positioned during the procedure because the intracranial internal carotid artery remained dilated even after withdrawal of the suture. The site of puncture was noted if the suture had perforated the intracranial internal carotid artery. Brains were prepared for histology by fixation in neutral buffered formalin for 7 days. The brains were sliced coronally to give two central consecutive 4-mm-thick slices. The tissue was processed with paraffin, and sections were stained with hematoxylin and eosin and examined by light microscopy by one of us who was unaware which procedure had been undertaken.

Results

In 18 control animals CBF and physiological variables were measured for up to 7 hours. CBF in the center of the MCA territory was 82 (95% confidence interval [CI], 80–84) ml/100 g per minute, and this together with the physiological variables was stable over a 7-hour period (Table 1).

Longa’s blunted suture method was attempted in 52 consecutive rats (group 1). Successful positioning of the suture was achieved in 29 rats (56%) (Figure 3). Mean CBF in the center of the MCA territory on the side of occlusion was 33 (CI, 28.3–37.7) ml/100 g per minute and in the control hemisphere was 72 (CI, 71–73) ml/100 g per minute. Failure occurred either as a result of perforation of the intracranial internal carotid artery or its branches or because the suture had been inserted as far as possible, but its tip lay short of the origin of the MCA.

Koizumi’s method was undertaken in the next 30 consecutive rats (group 2), and profound reduction of CBF was achieved in 28 animals (93%). In these rats mean CBF in the MCA territory on the side of the occlusion was 10.7 (CI, 9.9–11.5) ml/100 g per minute, and in the control hemisphere blood flows in the MCA territory were also reduced to 71 (CI, 70–72) ml/100 g per minute. These postintervention blood flows were in all cases significantly lower than those of controls \( (p<0.001) \), and CBF in the center of the MCA territory in group 2 was also significantly lower than in group 1 \( (p<0.001) \) (Figure 4). In all rats CBF and physiological variables were stable (Table 2).
TABLE 1. Cerebral Blood Flow and Physiological Variables in Control Rats

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF (ml/100 g per minute)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L MCA A</td>
<td>77±1.5</td>
<td>79±2.3</td>
<td>78±3.2</td>
<td>81±3.4</td>
<td>82±3.4</td>
</tr>
<tr>
<td>L MCA B</td>
<td>67±1.6</td>
<td>69±2.6</td>
<td>69±3.3</td>
<td>65±3.2</td>
<td>65±4.4</td>
</tr>
<tr>
<td>R MCA A</td>
<td>80±1.4</td>
<td>81±3.1</td>
<td>80±3.2</td>
<td>82±3.0</td>
<td>78±5.4</td>
</tr>
<tr>
<td>R MCA B</td>
<td>68±1.5</td>
<td>68±2.6</td>
<td>70±4.2</td>
<td>68±4.2</td>
<td>65±4.1</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>94±2.0</td>
<td>91±5.0</td>
<td>89±4.2</td>
<td>92±6.2</td>
<td>95±6.4</td>
</tr>
<tr>
<td>PCO₂ (kPa)</td>
<td>5.4±0.2</td>
<td>5.4±0.4</td>
<td>5.7±0.8</td>
<td>5.2±0.8</td>
<td>4.4±0.6</td>
</tr>
<tr>
<td>PO₂ (kPa)</td>
<td>16±0.8</td>
<td>15±1.2</td>
<td>13±1.5</td>
<td>16±3.2</td>
<td>14±1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.37±0.01</td>
<td>7.27±0.04</td>
<td>7.30±0.06</td>
<td>7.34±0.02</td>
<td>7.40±0.04</td>
</tr>
</tbody>
</table>

Values are mean±2 SEM.
CBF, cerebral blood flow; L, left; R, right; MCA, middle cerebral artery; A, center of MCA territory; B, posterior margin of MCA territory; MABP, mean arterial blood pressure.

Histology of the brain in those animals with mean postintervention CBF of <25 ml/100 g per minute revealed unilateral ischemic changes in the MCA territory. These consisted of shrunken, hyperchromatic neurons showing pericellular vacuolation, and in some cases there were discrete areas of infarction.

Discussion

In this article two techniques for producing MCA occlusion and thereby acute focal cerebral ischemia are compared. In our laboratory we have found Longa's method to be unreliable. Occlusion was attempted in 52 animals, but it was successful in only 29, and in only seven of these was CBF in the MCA territory reduced to <16 ml/100 g per minute. For occlusion to be successful, blood flow to the MCA from the anterior cerebral, internal carotid, and posterior communicating arteries must be interrupted. In Longa's method it is more difficult to pass the thread, and even when it is correctly positioned we do not think that flow around the thread is as effectively prevented as when Koizumi's thread is used. Thus, some flow is possible from the posterior communicating artery and from perforating vessels. Despite this, it is rarely possible to pass a larger-diameter 3-0 thread, and attempts to do so result in an even higher incidence of perforation of the vessels. The high rate of vessel perforation that we experienced using a 4-0 thread (10 of 52) is due to attempts to advance the thread against resistance when measured CBF shows little change. Longa, who reported only two instances of vessel perforation in his series of 71 occlusion experiments, postintervention CBF of <25 ml/100 g per minute.
sions, did not measure CBF during the attempted occlusion because they assessed the results of the procedure by neurological and histopathological methods. In Koizumi’s method silicone coating of the distal 5 mm of the thread gives it a soft and malleable coating, which gently dilates the vessels through which it passes. This leads to a greatly reduced risk of vessel perforation and a much tighter fit, preventing any flow around the thread. Although the diameter of the thread is greater, it is soft enough to allow it to pass into the anterior cerebral artery and thus occlude flow from this source. Our results are very similar to those reported in the original report, in which successful occlusion was reported in 55 of 60 animals (92%).5

In conclusion, both methods of MCA occlusion without craniectomy can produce profound reduction in CBF sufficient to produce focal ischemic changes in the MCA territory. We have found that Koizumi’s silicone-coated thread is able to produce ischemia more reliably and that reduction in CBF is more profound. We believe that this is the method of choice to study acute experimental ischemia in the MCA territory. The ability and minimally invasive nature of the method also make it most suitable for the study of chronic ischemia.

References

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**Editorial Comment**

When Ginsberg and Busto1 published their extensive review of rodent models of cerebral ischemia in December 1989, all middle cerebral artery (MCA) occlusion models discussed by these authors necessitated surgical exposure of this vessel. Nevertheless, in January of the same year, in the same journal, Longa and colleagues2 had described...
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