Reversible Middle Cerebral Artery Embolization in Dogs Without Intracranial Surgery

Phillip D. Purdy, MD, Michael D. Devous Sr., PhD, Charles L. White III, MD, H. Hunt Batjer, MD, Duke S. Samson, MD, Kirk Brewer, BS, and Kurt Hodges, BS

Using dogs, we developed an intravascular model for reversible middle cerebral artery occlusion that does not involve intracranial surgery or enucleation. Using silicone plastic plugs with a suture embedded within them, we embolized the middle cerebral artery in 19 dogs via the cervical carotid artery. The free end of the suture remained accessible in the neck, and after variable dwell times traction was placed on the suture and the plug was withdrawn. Placement of the plug in the middle cerebral artery produced ischemia in the basal ganglia. The degree and distribution of cortical ischemia were variable as evidenced by the pathologically documented scattered nature of infarcts that resulted when the plug was left permanently in the middle cerebral artery and when it was removed after 1 or 2 hours. Angiography demonstrated occlusion of the middle cerebral artery with the plug in place as well as reperfusion when the plug was withdrawn. This modification of a previously described model of middle cerebral artery occlusion provides an opportunity to study structural, physiologic, and biochemical events occurring in acutely hypoperfused cerebral tissue as well as critical changes leading to irreversible injury without the disadvantages of surgical manipulation required by all previous models of reversible cerebral ischemia. (Stroke 1989;20:1368-1376)

The creation of reversible cerebral ischemia offers the opportunity to study the sequence of events leading to infarction and will help identify the events most critical in determining irreversible tissue injury. Models involving creation of infarcts without reversal of ischemia do not permit differentiation of potentially reversible events from those that signify cell death. All previous models of reversible cerebral ischemia involved violation of the dura and direct mechanical manipulation of the middle cerebral artery (MCA), with attendant complications imposed by brain retraction, by infection, by leakage of cerebrospinal fluid, and by the necessity of enucleation. Molinari first described an intravascular model for the production of infarcts using silicone plugs injected into the cervical internal carotid artery and carried by the blood flow into the MCA. We have created a model using similar plugs with suture material embedded in the silicone cement. This provides a mechanism by which the plug can be delivered to the MCA, allowed to dwell for a variable period determined by the experimenter, and then removed, restoring blood flow to the MCA.

Materials and Methods

The plug is constructed using a standard 20-gauge venous catheter; 5-0 silk suture is passed into the tip of the catheter, looped at the hub, and passed back out the tip (Figure 1). The loop forms the event distal end of the plug, and doubling the suture prevents it from pulling out of the cement during withdrawal.

"Cement" is created using silicone rubber cement available in many brand names at hardware stores. Standard barium powder used in gastrointestinal radiology is mixed with the cement to produce radiopacity. The barium increases the cement's viscosity, but the cement retains its fluid characteristics.

Using a 3-ml syringe, the cement-barium mixture is injected into the hub of the suture-containing catheter, keeping the loop sticking out the hub until cement is seen coming from the tip of the catheter. After removing the syringe from the hub and filling...
FIGURE 1. Plug construction. Top: 5-0 silk suture is passed from tip to hub of 20-gauge catheter and looped. Middle: Barium-impregnated silicone cement is then injected into catheter hub until cement passes out catheter tip. Leaving hub filled with cement, suture is pulled into hub and more cement is injected. Injection pressure causes suture to squeeze out tip of catheter, pulling looped end of suture into catheter. Black suture can be seen in catheter (contrasted to cement), and when loop is in midpoint of catheter, suture/cement plug is set aside to dry for 24 hours. Bottom: Under magnification, catheter is subsequently dissected away from suture/cement plug it contains, being careful not to cut suture during dissection. Plug length is established by separating suture ends, cutting free end, and peeling away excess cement.

it with cement, the doubled suture is pulled slowly out the tip of the catheter until the loop is in the hub such that reapplication of the syringe to the hub will not engage the loop, which prevents the loop from passing into the catheter. By injecting more cement into the hub of the catheter, the suture is then carried further into the catheter until the loop can be seen at about the midpoint of the catheter. The catheter-suture-cement combination is then set aside and the cement is allowed to dry for 24 hours.

After the cement dries completely, the catheter is dissected from the plug using a magnification lamp and a No. 11 scalpel. This produces a plug of a length that can be revised by pulling the ends of the suture apart and cutting off the shorter end. We used plugs 7 mm long in most dogs in this study. Any residual cement can be peeled from the free end of the suture, which serves as the retraction suture after the plug is delivered.

The plug is delivered using a 16-gauge venous catheter (Figure 2). The free end of the suture is inserted into the tip of the catheter until the plug is in the tip and the suture passes out the hub. The suture is then coiled in a 12-ml syringe such that the free end of the suture passes out the barrel of the syringe. The syringe is then attached to the catheter. Saline is poured into the barrel of the syringe while placing a finger over the catheter tip to prevent the plug from extruding and while exercising caution to maintain the free end of the suture out the barrel of the syringe. The plunger is then placed in the syringe, again maintaining a finger over the catheter tip, such that the plunger now holds the free end of the suture against the barrel of the syringe. This prevents the entire suture from being injected into the artery when the plug is delivered. Interventional radiologists will recognize this assembly as analogous to that commercially available in flow-directed balloons. When the saline is injected, it carries the plug-suture combination into and along the course of the vessel.

Nineteen mongrel dogs, unselected as to sex, were anesthetized with approximately 15 mg/kg i.v. thioamylal. Following intubation and maintenance on artificial ventilation with room air, the dogs' necks were shaved and their carotid arteries were surgically exposed at the level of the bifurcation. The left common, internal, and external carotid arteries were identified. Umbilical tape was placed around the left common carotid artery, and 2-0 suture was placed around the left internal and external carotid arteries. Following surgical exposure of the right femoral artery in the groin, a 5.5-French Hinck 1 catheter (Cook, Inc., Bloomington, Indiana) was inserted and advanced under fluoroscopic observation to the left vertebral artery. This catheter was subsequently used for verification angiography.

Following fluoroscopic verification of catheter placement and intravenous administration of 5,000 units of heparin, an incision approximately 2 mm long was made in the left internal carotid bulb distal to the left common carotid artery bifurcation. The 16-gauge catheter/12-ml syringe loaded with a plug
Stroke Vol 20, No 10, October 1989

FIGURE 2. Plug delivery. Using 16-gauge venous catheter, plug is loaded such that it is at catheter tip, with suture extending out hub. Top: Suture is then coiled in 12-ml syringe, with free end held against barrel by plunger (arrow), which prevents entire suture from being delivered. Syringe is filled with water or saline. Plug-containing catheter is then placed directly into internal carotid artery. When injected with moderate force, plug is carried into middle cerebral artery, where it can be left in place or withdrawn at will. Dog is heparinized to prevent distal thrombosis. Bottom: Plug/suture after injection from catheter.

was then inserted through the incision, and the suture around the left internal carotid artery was used to tie the vessel off proximal to the catheter to prevent blood loss during the procedure. All traction on the left common and external carotid arteries was removed, and the plug was delivered by moderate force on the syringe plunger.

Immediately following plug delivery, positioning was confirmed via plain-film radiography (Figure 3, top) and angiography (Figure 3, left). The quality of the vertebral angiograms was enhanced if both common carotid arteries were temporarily clamped during angiography. This eliminated most collaterals and resulted in better filling of the entire circle of Willis.

Following angiographic verification of placement, the plugs were left in place for 5 (n=1), 20 (n=1), 30 (n=1), 60 (n=5), or 120 (n=6) minutes or 48 hours (permanently, n=5). Removal of plugs that were removed was via traction on the free end of the suture retained in the neck. Reflow into the MCA was verified in all dogs by repeat angiography following plug removal (Figure 3, right). The catheter was then removed from the neck and the wounds were sutured. The dogs were allowed to awaken and, when adequate spontaneous ventilation was demonstrated, they were extubated and returned to the recovery area of our Animal Resources Center. Dogs were examined at 24 and 48 hours after embolization for evidence of hemianopsia, hemiparesis, circling behavior, and for evidence of diminished level of consciousness. All dogs were killed at 48 hours by pentobarbital overdose. Following sacrifice, all brains were removed and placed in 10% phosphate-buffered neutral formalin. After at least 48 hours of immersion-fixation, the brains were sliced at 3-mm intervals in the coronal plane and photographed. Slices were then dehydrated in alcohol and xylene, embedded in paraffin, mounted on oversized glass slides, stained with hematoxylin and eosin, coverslipped, and examined with a light microscope.

Results

Results are summarized in Table 1. In all dogs successfully embolized, it was possible to remove the plugs from the MCA and verify reflow angiographically. In dogs from which the plugs were removed after 5, 20, or 30 minutes, no infarcts were seen. Dogs with dwell times of 60 or 120 minutes evidenced mixed results; some brains were histologically normal and some evidenced infarction (Figure 4). All dogs with permanent placement (Figure 5) showed cortical infarction in the MCA territory, though the infarcts varied in size and location. We are unsure why Dog 16 showed apparent anterior cerebral artery distribution infarction. Basal ganglia infarction (Figure 6) was seen in all but one dog with permanent placement; this dog (Dog 17) was the only one in which the plug lodged in the distal MCA, beyond the lenticulostriate arteries. Review of Dog 17’s arteriogram revealed some filling of the most proximal MCA. In other dogs with cortical infarcts, pathologic verification of plug placement was unavailable since the plug had been removed. It is interesting that no dog with basal ganglia infarction was without cortical infarction, but the converse is not true. This may reflect collateralization to the basal ganglia from the posterior circulation, though the possibility of more distal plug placement in these dogs could not be excluded, even with more proximal angiographic occlusion. In no dog with plugs constructed as
FIGURE 3. **Top:** Radiograph of dog shows plug (arrow) in middle cerebral artery (MCA), which runs perpendicular to plug. Density nearly aligned with axis of arrow is bony. **Bottom:** Angiograms demonstrate (left) MCA occlusion by plug and (right) subsequent reflow following plug removal. Note mild vasodilation of MCA when plug was removed, as well as retrograde blood flow in internal carotid artery, which was tied off proximally. Some degree of vasodilation was common in MCA immediately following plug removal. Vertebral angiography was found to be superior to carotid angiography to demonstrate circle of Willis using temporary bilateral occlusion of common carotid arteries during angiography.
TABLE 1. Summary of Results of Reversible Middle Cerebral Artery Embolization in Dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Histology</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>?Hemiparetic</td>
</tr>
<tr>
<td>20 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cortical infarct</td>
<td>Hemiparetic</td>
</tr>
<tr>
<td>5</td>
<td>Not done</td>
<td>Dead</td>
</tr>
<tr>
<td>6</td>
<td>Basal ganglia and cortical infarct</td>
<td>Dead</td>
</tr>
<tr>
<td>7</td>
<td>Cortical infarct</td>
<td>Normal</td>
</tr>
<tr>
<td>8</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>120 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Normal</td>
<td>?Hemiparetic, circling</td>
</tr>
<tr>
<td>10</td>
<td>Normal</td>
<td>Hemiparetic</td>
</tr>
<tr>
<td>11</td>
<td>Basal ganglia and cortical infarct</td>
<td>Comatose</td>
</tr>
<tr>
<td>12</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>13</td>
<td>Cortical infarct*</td>
<td>Normal</td>
</tr>
<tr>
<td>14</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Permanent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Basal ganglia and cortical infarct</td>
<td>Normal</td>
</tr>
<tr>
<td>16</td>
<td>Basal ganglia and cortical infarct</td>
<td>Hemiparetic, circling, hemianopsia</td>
</tr>
<tr>
<td>17</td>
<td>Cortical infarct†</td>
<td>Hemiparetic, circling, hemianopsia</td>
</tr>
<tr>
<td>18</td>
<td>Basal ganglia and cortical infarct</td>
<td>Dead</td>
</tr>
<tr>
<td>19</td>
<td>Basal ganglia and cortical infarct</td>
<td>Circling</td>
</tr>
</tbody>
</table>

Unless noted, all cortical infarcts were in middle cerebral artery (MCA) territory (lateral or sylvian cortex) and all plugs lodged in proximal MCA, allowing occlusion of lenticulostriate arteries, which accounts for basal ganglia infarcts in all but one dog with permanent placement.

*Small infarct in anterior cerebral artery territory (medial cortex superiorly).
†Plug lodged in MCA distal to sylvian fissure. Infarct occurred in cortex adjacent to plug.

Described above did the suture pull out and leave the cement behind.

Overall, dogs with larger infarcts evidenced worse neurologic results. We are uncertain why the behavior in Dog 15 was judged to be normal in the face of characteristic infarction caused by permanent plug placement; this was the only neurologically unremarkable examination among the permanent placement dogs.

Discussion

The ability to deliver a reliable insult to a reproducible region is crucial to investigation at a cellular or biochemical level of the events surrounding the onset of ischemia and subsequent infarction. Though surgical models reliably create basal ganglia ischemia, the proximity of the basal ganglia to the MCA places the area of infarction potentially within the operative field, suggesting that the injury to the basal ganglia may be an artifact of surgery. In MCA occlusions, the cortical insult is reliable in its presence but not in its location or extent. If the insult is great enough to create large cortical infarcts, they are largely fatal. Additionally, the accompanying enucleation and dural violation with these models complicates clinical follow-up of these animals due to the potential for meningitis and the imposition of visual abnormality. It therefore detracts from these models' applicability to transient or permanent ischemia in humans.

The use of cement plugs has been shown to create reproducible ischemia, but the insult was not reversible and the model was therefore unable to distinguish reversible from irreversible insults. The removable plug model represents an intravascular mechanism for delivery of an insult for a fixed time that can then be reversed at the discretion of the experimenter. This model involves no cranial surgery, and even when the plug remains in place the insult is survivable, which means that clinical evaluations across the entire temporal spectrum of experimentally induced insults are possible with this model.

In addition to the advantages of reversibility and survivability, the anatomic reliability of the basal ganglia insult with proximal MCA placement in the cement plug model, as shown in this and other studies, offers a region of the brain that can be identified prospectively and studied to establish the critical metabolic events that signify continued reversibility as opposed to obligatory cell death. This may
be helpful in designing informed therapies to modify metabolism at points crucial to preserving neuronal survival. This could include trials to observe whether a treatment altered the dwell time needed before plug placement resulted in infarction. Further studies are needed to better characterize the time course of events following plug placement and withdrawal.

In attempting embolization, multiple trials were unsuccessful when traction was placed on the common or external carotid arteries during injection of the plug. In one dog, dissection following unsuccessful embolization revealed the plug in the maxillocarotid anastomotic vessel. We believe that preservation of antegrade external carotid blood flow is helpful to direct the plug into the MCA rather than into the maxillocarotid anastomosis. Though this was not specifically studied, the importance of maintenance of systemic blood pressure in the external carotid circulation was discussed by Molinari in his original description. We also

![Figure 4. Photomicrographs of brain from Dog 4. Top: Pallor due to rarefaction and edema in deep cortex and subcortical white matter from parieto-occipital region, indicative of recent ischemia. Hematoxylin and eosin stain ×44. Bottom: Several necrotic deep cortical neurons (arrows) in rarified neuropil. Hematoxylin and eosin stain, ×437.](image-url)
believe that longer plugs are a hindrance to success, though the effect of plug length has not yet been studied independent of the effect of common or external carotid artery traction. Plug placement in many dogs was initially variably successful. However, using large dogs (>25 kg), we were successful in 14 of the last 18 in which we attempted placement.

We did not control factors such as blood oxygenation and systemic arterial blood pressure. These factors may contribute to the heterogeneity of results for the 60- and 120-minute dwell time dogs. If so, controlling these factors might allow more precise establishment of a critical time separating infarction from preservation. This model may also be appropriate for studying the precise role these factors play in cerebral infarction to help guide future therapies.

References
FIGURE 6. Photomicrographs of brain from Dog 19. Top: Section of basal ganglia demonstrating loss of tissue cohesion and early acute inflammatory cell infiltration, indicative of recent ischemic necrosis. Hematoxylin and eosin stain, x218. Bottom: Necrotic neuron (arrow) among capillaries with prominent, reactive endothelial nuclei. Hematoxylin and eosin stain, x437.


**KEY WORDS** • animal models • cerebral ischemia • dogs
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Stroke. 1989;20:1368-1376
doi: 10.1161/01.STR.20.10.1368

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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