New Model of Cerebral Thrombosis in Dogs

M. Hirschberg, MD, and B. Hofferberth, MD

Experimental in situ thrombosis of the middle cerebral artery was produced in dogs by use of intravasally placed copper coils, which subsequently gave rise to an obstructing autologous thromboembolus. The resulting thrombosis was produced in the middle or rostral cerebral artery within 5–15 minutes after delivery of the thrombogenic device. The correlation of location of the coil in the intracranial vasculature of the internal carotid artery with the anatomic distribution of resulting cerebral infarcts suggests that this experimental model can produce a selective acute local occlusion of cerebral vessels in a high proportion of dogs without violating the cranium. The composition of the autologous thromboembolus, the low mortality rate, and the excellent reproducibility will make the correlative study of thrombolytic agents and their therapeutic efficacy possible. (Stroke 1988; 19:741–746)

A n effective regimen for the treatment of acute thrombotic cerebral ischemia remains elusive despite the testing of many theoretically promising compounds. Rational therapeutic approaches should be directed primarily toward lysis of thrombus and restoration of tissue perfusion. Clinical trials of thrombolytic agents administered late in the development of stroke have reported no clinical benefit or hemorrhagic complications. Recent production of new, thrombus-specific thrombolytic agents such as tissue plasminogen activator (tPA), single-chain urokinase plasminogen activator (scuPA), or acylated derivatives of the streptokinase-plasminogen complex warrants the development of appropriate animal models of evolving cerebral thrombosis in situ to study the effects of those drugs in cerebral thrombotic stroke and, possibly, in the salvage of cerebral tissues. In spite of a wide variety of brain models that have been developed to study focal ischemic brain damage and several models for study of thrombolytic agents in stroke, there is none that simulates adequately the condition in which thrombosis within a cerebral vessel produces a gradual, well-defined, and potentially reversible obstruction of the lumen.

We describe experimental focal cerebral ischemia produced in dogs by placing a copper coil into the peripheral internal carotid artery (ICA) system using an extracranial technique based on studies of experimental thrombosis induced in the coronary arteries. The fate and constitution of the emboli, the role of arterial width, and the subsequent cerebral infarctions were studied clinically and pathologically.

Materials and Methods

Seventeen healthy adult mongrel dogs of either sex weighing 12–16.5 kg were studied (Table 1). Analgesia was induced with 25 mg/kg i.v. sodium pentobarbital and maintained with subsequent doses as necessary and 75% nitrous oxide-25% oxygen. Pancuronium bromide (40 mg i.v.) was given to facilitate tracheal intubation. A respirator delivered a tidal volume of 15–20 ml/kg, and its rate was adjusted on the basis of $\text{Paco}_2$, $\text{PaO}_2$, and arterial blood pH. Body temperature was maintained at 37° C using a thermal blanket. Two intravenous catheters were inserted percutaneously for drug and fluid administration and venous blood sampling. Systemic arterial blood pressure was obtained using a Statham strain gauge (Cleveland, Ohio) and registered on a Sirsec monitoring system (Siemens, Berlin, F.R.G.). Arterial blood was sampled through the same catheter.

Technique of Embolization

To keep interference with the clotting and fibrinolytic systems as low as possible, surgical measures were kept to a minimum. A 5-French, thin-walled polyethylene cerebral catheter (Medisco, Ottmarsbohlt, F.R.G.) was injected coaxially into one femoral artery through an introducer system (Cook Europe, Bjaeverskov, Denmark). After preliminary recordings of the cardiac and respiratory rates and the arterial blood pressure and after analysis of blood gases, the catheter was advanced into the right or left ICA under fluoroscopy. The vessel was identified by injecting small amounts (1.5–2 ml) of nonionic radiopaque contrast media (Ultravist 300, Schering, Berlin, F.R.G.). After identification of the ICA and demonstration of the circle of Willis, the catheter was fixed in position.

The thrombogenic devices were 1.0 mm o.d. helixes of solid copper wires (0.125 mm in diameter). Five turns of the wire were used so that the copper coil was 3.0 mm long.

In 14 of the 17 dogs, the thrombogenic device was pushed through the catheter up to the tip by means of a guide wire. After withdrawal of the guide wire, the copper coil was flushed into the distal ICA using 5 ml physiological saline. From there the coil floated into the ipsilateral middle cerebral artery (MCA) or the rostral cerebral artery (RCA). Delivery of the copper coil was confirmed by image intensifier control. Angiography was performed every 5 minutes to assess the time of thrombotic occlusion of the vessel.
Three precautions were used during the procedure: slow, gentle introduction of the catheter without a guide wire to prevent perforation of the vessels; continuous physiological saline perfusion of the catheter to prevent same way, but copper coils were not introduced. slow, gentle introduction of the catheter without a guide wire to prevent perforation of the vessels; continuous physiological saline perfusion of the catheter to prevent same way, but copper coils were not introduced. 

After 3 hours of vascular occlusion (thrombotic occlusion of the coils) three of the 14 study group dogs were studied further. During our study the method of delivering coils into cerebral circulation by this extracranial technique proved to be safe and easy to perform. It caused no immediate mortality.

### Results

After flushing the copper coil into the cerebral circulation, its final settlement could be observed by

### Table 1. Parameters in Dogs With Cerebral Thrombosis

<table>
<thead>
<tr>
<th>Dog</th>
<th>Body wt (kg)</th>
<th>MCA:RCA diameter ratio</th>
<th>Time until occlusion (min)</th>
<th>Duration of occlusion (hr)</th>
<th>Location of coil</th>
<th>Resulting infarct size (%)</th>
<th>Clinical rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.5</td>
<td>L 1.0</td>
<td></td>
<td>0</td>
<td>No coil</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>13.0</td>
<td>L 1.0</td>
<td></td>
<td>0</td>
<td>No coil</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>13.5</td>
<td>L 0.9</td>
<td></td>
<td>0</td>
<td>No coil</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Study group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15.0</td>
<td>R 1.1</td>
<td>10</td>
<td>3</td>
<td>Distal R MCA</td>
<td>v</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>15.0</td>
<td>L 1.0</td>
<td>15</td>
<td>3</td>
<td>Proximal L MCA</td>
<td>v</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>16.5</td>
<td>L 1.1</td>
<td>10</td>
<td>3</td>
<td>Proximal L MCA</td>
<td>v</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>13.5</td>
<td>R 1.0</td>
<td>10</td>
<td>12</td>
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<td>c, w</td>
<td>36.7</td>
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<td>10</td>
<td>11–12</td>
<td>Proximal L MCA</td>
<td>b, c, w</td>
<td>18.9</td>
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<td>9</td>
<td>14.5</td>
<td>R 1.0</td>
<td>5</td>
<td>12</td>
<td>Proximal R MCA</td>
<td>c, w</td>
<td>9.6</td>
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<tr>
<td>10</td>
<td>14.0</td>
<td>L 0.8</td>
<td>15</td>
<td>11–12</td>
<td>Proximal L MCA</td>
<td>b, c, w</td>
<td>28.0</td>
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<tr>
<td>11</td>
<td>16.0</td>
<td>L 1.0</td>
<td>15</td>
<td>12</td>
<td>Proximal L MCA</td>
<td>b, c, w</td>
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<tr>
<td>12</td>
<td>14.5</td>
<td>L 1.0</td>
<td>10</td>
<td>12</td>
<td>Proximal L MCA</td>
<td>b, c, w</td>
<td>21.7</td>
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<tr>
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<td>10</td>
<td>12</td>
<td>R RCA</td>
<td>c, w</td>
<td>ND</td>
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<tr>
<td>13b</td>
<td>14.0</td>
<td>L 1.0</td>
<td>10</td>
<td>12</td>
<td>Distal L MCA</td>
<td>b, c, w</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>15.0</td>
<td>R 1.1</td>
<td>5</td>
<td>12</td>
<td>Proximal R MCA</td>
<td>b, c, w</td>
<td>35.6</td>
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<tr>
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<td>16.0</td>
<td>L 1.2</td>
<td>15</td>
<td>12</td>
<td>Proximal L MCA</td>
<td>b, c, w</td>
<td>21.0</td>
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<tr>
<td>16</td>
<td>15.0</td>
<td>L 1.1</td>
<td>10</td>
<td>12</td>
<td>Proximal L MCA</td>
<td>b, c, w</td>
<td>23.0</td>
</tr>
<tr>
<td>17</td>
<td>15.0</td>
<td>L 1.0</td>
<td>5</td>
<td>12</td>
<td>Proximal L MCA</td>
<td>c, w</td>
<td>15.6</td>
</tr>
</tbody>
</table>

All coils had outside diameter of 1.0 mm. L, left; R, right; MCA, middle cerebral artery; RCA, rostral cerebral artery; ND, not determined; v, congestion of vessels; c, cortical involvement; w, white matter involvement; b, basal ganglia involvement.

Three precautions were used during the procedure: slow, gentle introduction of the catheter without a guide wire to prevent perforation of the vessels; continuous physiological saline perfusion of the catheter to prevent its thrombotic occlusion and subsequent microembolization of peripheral cerebral vessels; and slow, low-pressure injection of contrast media to prevent opening of the acutely occluded coils.

Three dogs serving as controls were treated in the same way, but copper coils were not introduced.

In the remaining 11 study group dogs the neurologic deficit was rated 6 hours after restitution of spontaneous respiration according to a modification of the criteria defined by Smith and coworkers: 0, no neurologic deficit; 1, circling, single extremity weakness; 2, marked hemiparesis but walking possible; 3, animal awake but not able to move; or 4, spontaneous death. After 12 hours, the nine surviving study group dogs were deeply anesthetized with 25 mg/kg i.v. sodium pentobarbital and a central analgesic (0.15 mg i.v. fentanyl hydrochloride) and were killed.

All the brains were removed and fixed in 4% formalin. In each dog the copper coil was located in the cerebral circulation by pathologic examination or image intensifier control. diameters of the origin of the MCA and RCA were measured with a micrometer.

After fixation, the brains were cut in 5-mm coronal slices, embedded in paraffin, and sectioned for staining with hematoxylin and eosin (HE) to demarcate the area of ischemic pallor. In these slices, the area of largest infarct extent was analyzed macroscopically and, using a digitizing board, interfaced to a computer (Apple Computers). The area of infarction was calculated as percent of the total cross-sectional area of the hemisphere. All areas were measured three times, and the measurements agreed to within 5%. Only brains with a copper coil in the MCA were studied further. Histopathologic evaluation of the slices was performed according to well-established criteria. Additional sections were stained with phosphotungstic acid and hematoxylin (PTAH) to exclude the presence of thromboemboli or fragmented clots in the distal cerebral circulation.

In representative brains, the occluded vessel with the thrombus and the copper coil were removed and fixed in 4% formalin for histology (HE staining). These tissues were embedded in methacrylate and cut on a special microtome.

**Results**

During our study the method of delivering coils into the cerebral circulation by this extracranial technique proved to be safe and easy to perform. It caused no immediate mortality.

After flushing the copper coil into the cerebral circulation, its final settlement could be observed by
image intensifier control, and its location in the MCA or RCA could be identified from a plain x-ray of the skull in the ventrodorsal position (Figure 1). As confirmed by pathologic examination, location of the radiopaque thrombogenic device in the anterior midline indicated its deposition in the RCA, whereas a copper coil anterolaterally could be confirmed as lodging in the MCA. Fourteen of 15 coils settled primarily in the MCA territory (Table 1). Only in Dog 13 was the coil observed to flow with the blood into the right RCA first (13a). In a second procedure in Dog 13, the catheter was changed to the contralateral ICA and a second coil was delivered into the left MCA (13b). No coils were detected in the posterior cerebral circulation. All coils were ipsilateral to the catheter.

On pathologic examination deposition of the coils in the trunk of the MCA proximal or distal to its trifurcation was assessed. In 11 of the 14 study group dogs, the coil could be identified proximal to the trifurcation of the MCA (Table 1). The anatomic position of the copper coil in the MCA is shown in Figure 2 as a representative case. The copper coil in the RCA was found just beneath the knee of the RCA around the corpus callosum.

Measurement of diameters of the MCA and RCA where they branch from the circle of Willis revealed that the mean diameter of the MCA was larger than that of the RCA. This facilitated the passage of emboli primarily into the MCA. The ratio of the diameters is shown in Table 1. Only in Dogs 3, 10, and 13a was the RCA diameter larger than that of the MCA. In Dog 13, the ratio of the diameters favored the RCA only on the right side. Angiography performed every 5 minutes showed that occlusion of the coils, and thus of the vessels, occurred within 5–15 minutes after delivery of the coils into the cerebral circulation (Table 1).

Histologic examination of the thrombi that accumulated within the copper coils as well as in the descending and ascending parts of the vessels (apposition thrombus) revealed that thrombotic material in the coil consisted of fibrin, platelets, and erythrocytes in the form of a mixed platelet-fibrin thrombus. In the apposition thrombus, the relative content of erythrocytes was higher.

Each study group dog was severely affected by embolization of the MCA (Table 1). All were slow to awaken from anesthesia; two (Dogs 8 and 10) died spontaneously between 11 and 12 hours after vessel occlusion. Only one (Dog 9) exhibited a neurologic deficit rated as 2, whereas the remaining eight dogs received a score of 3, indicating that they were unable to move. In the control dogs, no signs of cerebral infarctions could be detected.
In the three study group dogs with acutely evolving infarction (those killed 3 hours after thrombotic occlusion of the MCA), no gross tissue changes except swelling and congestion of cerebral vessels ipsilateral to the copper coil could be detected on pathologic examination. The histopathology of the brains showed no neuronal changes consistent with the criteria of Little and coworkers. An area of ischemic pallor could not be identified.

After 12 hours of MCA or RCA occlusion in the 11 remaining study group dogs, pathologic changes were more overt. As demonstrated in Table 1, the changes always occurred in the brain areas supplied by the occluded arteries. The brains disclosed swelling of the infarcted hemispheres. In these areas, capillaries and arterioles were packed with erythrocytes. After HE staining, the affected cerebral areas could be identified readily because of decreased staining. They showed histologic changes in the form of moderate to severe neuronal shrinkage, cytoplasmic eosinophilia, and nuclear pyknosis. The area of infarction included the basal ganglia, cortex, and white matter in eight of the 11 dogs. As shown in Table 1, infarct size in these eight dogs ranged from 18.9% to 46.0% (mean ± SD 27.7 ± 9.8%) of the affected hemisphere. In seven of these eight dogs, the coils were situated proximal to the trifurcation of the MCA. Only in Dog 13 had the coil passed beyond the trifurcation and affected the basal ganglia, cortex, and white matter. Dog 13 was excluded from area analysis because of the contralateral occlusion of the RCA. In three dogs (Dogs 7, 9, and 17), the ischemic lesions were confined to cortical tissue and white matter. Area analysis revealed infarct sizes of 36.7%, 9.6%, and 15.6%, respectively. In two of these three (Dogs 9 and 17) the coil was located proximal to the MCA trifurcation and the infarct was small, whereas the infarct was relatively large (36.7%) in Dog 7, which had isolated cortical and white matter involvement due to a distally lodging coil. No lesions were detected in the border zones between the territories of the MCA and RCA in any dog. Cerebral hemorrhages were not observed. In the one dog with RCA occlusion, the area of infarction involved the RCA-supplied cortex and white matter of the frontal and parietal lobes. PTAH staining gave no evidence of fragmented clots in the distal cerebral circulation in any dog.

Discussion

In this model of cerebral ischemia, a single cerebral vessel was occluded selectively by a well-defined autologous thromboembolus. The resulting area of ischemic infarction involved deep and superficial cerebral tissues. Using the technique of copper coil occlusion of the MCA trunk proximal to its trifurcation,
we produced a lesion involving basal ganglia structures, cortex, and white matter in eight of 11 dogs. Area analysis demonstrated a rather homogeneous size of infarction in these dogs. When the coil went too far distally to occlude the lenticulostriate arteries (Dog 7), a relatively large area of cortical and white matter infarction was observed, probably as a result of peripheral undercutting of the collateral vessels. In spite of proximal occlusion of the MCA in two dogs (9 and 17), presumably due to variation of vascular supply and collateralization, basal ganglia structures were not affected and the infarct was rather small.

Similar results have been reported with models of intravascular embolization of the MCA with silicone cylinders.5-7 Experiments with the technique of ligation or clipping of the MCA have been reported to be unsuccessful in creating significant ischemic damage to the brain in dogs.13 This discrepancy between results of experimentally induced infarction was explained by the hypothesis that embolization of the MCA trunk by a thrombus or embolus long enough to occlude the orifices of the lenticulostriate and perforating arteries undercuts the blood supply sufficiently to cause infarction.7 When these orifices remain open (with the technique of proximal ligation or clipping of the MCA), some anastomoses can still reach the perforating or lenticulostriate arteries via the trapped MCA trunk.7 This is supported by the results from our experiments: in most dogs the copper coil caused complete MCA infarction by thrombus formation along the proximal MCA in the form of an ascending and descending apposition thrombus.

Histopathology showed marked alterations in the infarcted brains of dogs after up to 12 hours of MCA occlusion but not in dogs after 3 hours of ischemia. In the model presented here, an extracranial technique employing the femorocerebral approach without major surgery is introduced. This model has the advantage of sparing the arterial nerve supply and not interfering with the inflammatory and clotting systems, which are necessary conditions when testing the efficacy of thrombolytic agents.

On the other hand, all intravascular methods of embolization of cerebral vessels have the disadvantage that the site of vascular occlusion is controlled only by the size and physical characteristics of the emboli used.17 Our results show a high reproducibility of copper coil delivery; the majority of copper coils went into the MCA with the blood flow. The greater diameter of the MCA in relation to the RCA favored this course. Molinari10,14 demonstrated similar results in his experiments with silicone emboli. In a high proportion of our cases, the coils were situated proximal to the MCA trifurcation, leading to infarctions of equal extent in eight of 11 dogs. In all dogs, the clinical score indicated severe cerebral infarction.

Contrary to the results reported by Kordenat and coworkers,12 thrombi developed faster in our experiments, which may be due to the much smaller coils used. The copper coils caused well-defined local thrombosis of a single cerebral vessel whose fate under administration of thrombolytic agents can be studied angiographically. Studies under similar conditions in the coronary arteries have been done.14 Furthermore, fragmentation of the clots was not observed in our experiment, as demonstrated by PTAH staining. However, this may happen with autologous blood clots during injection, thus leading to multifocal, undefined infarction.17

In conclusion, this model of focal cerebral ischemia caused by a well-defined, autologous thrombus in a nonprimate with a spectrum of sensitivities to plasminogen activators similar to that of humans19 may prove useful for the correlative study of thrombolytic agents and their therapeutic efficacy.

References


**KEY WORDS** • cerebral embolism and thrombosis • dogs • cerebral infarction
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