Complications and Pitfalls in Rat Stroke Models for Middle Cerebral Artery Occlusion

A Comparison Between the Suture and the Macrosphere Model Using Magnetic Resonance Angiography

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Background and Purpose
Investigating focal cerebral ischemia requires animal models that are relevant to human stroke. Complications and side effects are common among these models. The present study describes potential pitfalls in 3 techniques for middle cerebral artery occlusion (MCAO) in rats using magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA).

Methods
Rats were subjected to temporary MCAO for 90 minutes using the suture technique (group I; n=10) or to permanent MCAO using the suture technique (group II; n=10) or the macrosphere technique (group III; n=10). Clinical evaluation was performed after 3 hours and 24 hours. After 24 hours, animals underwent MRI and MRA to determine lesion size and the intracranial vascular status.

Results
Hemispheric lesion volume was significantly smaller in group I (14.6%) compared with groups II (35.2%; P<0.01) and III (21.3%; P<0.05). Two animals (1 each in group II and III) did not demonstrate neurological deficits and had no lesion on MRI and a patent MCA main stem on MRA. Subarachnoid hemorrhage was detected in 2 animals (1 each in group I and II). MRA indicated a patent MCA main stem in 2 animals (group II), although both rats displayed neurological deficits. Hypothalamic infarction with subsequent pathological hyperthermia was detected in all animals in group II and in 1 rat in group III.

Conclusions
Model failures occurred frequently in all groups. MRI and MRA helps to identify animals that need to be excluded from experimental stroke studies. (Stroke. 2004;35:2372-2377.)

Key Words: hyperthermia ■ magnetic resonance angiography ■ magnetic resonance imaging ■ stroke

Animal models of focal cerebral ischemia allow study of stroke pathophysiology and evaluation of new therapeutic approaches. Among different animal models available for focal cerebral ischemia induction, endovascular techniques are less invasive and therefore preferable. Among the endovascular techniques for middle cerebral artery occlusion (MCAO), the suture occlusion technique in rats is the most frequently used method. In this model, a monofilament is advanced into the internal carotid artery (ICA) until it blocks blood flow to the middle cerebral artery (MCA). This technique provides reproducible MCA territory infarctions and allows reperfusion by retracting the suture. Permanent MCAO with the suture technique, however, has one important disadvantage: insertion of the suture occludes the entire course of the ICA, leading to obstruction of the hypothalamic artery (HA). This causes hypothalamic infarction with associated pathologic hyperthermia that confounds neuroprotective drug evaluation. The recently introduced macrosphere model has been developed to overcome this side effect by the intra-arterial embolization of 6 TiO₂ spheres that selectively block blood flow to the MCA main stem without obstructing the HA. This model avoids hypothalamic infarction and pathological hyperthermia but does not allow for reperfusion.

The present study was designed to evaluate validity, complications, and side effects of 3 different MCAO models (permanent MCAO using the suture and the macrosphere models and transient occlusion with the suture technique). MRI and MRA have been applied to identify model failures.

Materials and Methods

Animal Preparation
All procedures are in accordance with our institutional guidelines and the German animal protection legislation.
Thirty male Sprague-Dawley rats (290 to 350 grams; Harlan Winkelmann, Borchen, Germany) were anesthetized with 5% isoflurane delivered in air for 2 minutes. Anesthesia was maintained with 2% to 3% isoflurane delivered in air at 0.5 L/min during surgery. Body temperature was continuously monitored with a rectal probe and maintained at 36.5°C to 37.0°C.

The right external carotid artery (ECA) was ligated and transsected to create an ECA stump with a length of ~5 mm. The pterygopalatine branch of the ICA was also occluded.

**Suture Models**

A 4-0 silicone–coated nylon suture was introduced through the ECA stump as described previously.4 The occluder was advanced into the ICA 16 to 18 mm beyond the carotid bifurcation until mild resistance indicated that the tip was lodged in the anterior cerebral artery and thus blocked blood flow to the MCA. Reperfusion was induced in group I by removing the suture 90 minutes after MCAO.

**Macrosphere Model**

PE-50 tubing, filled with saline and 6 TiO2 macrospheres (diameter, 0.315 to 0.355 mm; BRACE GmbH, Alzenau, Germany), was inserted into the ECA stump. The tip of the tubing was placed in the carotid bifurcation without affecting the blood flow to the ICA. Then the macrospheres were advanced separately into the ICA by a slow injection of ~0.05 mL saline each, until they were moved passively into the cerebral circulation by the blood flow. After macrosphere injection, the ICA was carefully flushed with 0.5 mL saline.5

**Experimental Protocol**

Thirty animals were randomly subjected to group I to III: (1) group I, MCAO for 90 minutes, followed by reperfusion (suture model; n = 10); (2) group II, permanent MCAO (suture model; n = 10); and (3) group III, permanent MCAO (macrosphere model; n = 10).

The following exclusion criteria were used: no neurological deficits 3 hour after MCAO (score 0); occlusion of the MCA main stem on MRA after 24 hours in the reperfusion group; MCA main stem not being occluded on MRA after 24 hours in groups II and III; no infarction in the MCA territory on T2-weighted MRI and stem not being occluded on MRA after 24 hours in groups II and III; no infarction in the MCA territory on T2-weighted MRI and stem not being occluded on MRA after 24 hours in groups II and III; no infarction in the MCA territory on T2-weighted MRI and stem not being occluded on MRA after 24 hours in groups II and III.

**Postmortem Analysis**

After MRI, the animals were deeply anesthetized and euthanized. The brains were removed and the localization of the macrospheres in the basal cerebral arteries was determined using a magnifying glass. The brains were sectioned coronally into 6 slices (thickness, 2 mm), incubated in a 2% solution of TTC at 37°C, and then examined for the presence of TTC-positive (white) areas. The position of the midline was determined using neuronal landmarks as described previously.7 Lesion volumes were determined by computer-aided manual tracing of the hyperintense lesions and corrected for the space-occupying effect of brain edema as described previously using the following equation:

\[
\text{VLC} = \frac{\text{HCc} - \text{HVc} + \text{LVu}}{\text{HVc}} \times 100
\]

where \(\text{VLC}\) indicates edema–corrected lesion volume (in percent of the hemispheric volume); \(\text{HCc}\) and \(\text{HVc}\) indicate contralateral and ipsilateral hemispheric volume; and \(\text{LVu}\) indicates uncorrected lesion volume.

**MRA**

Arterial MRA was acquired using a 2-dimensional time-of-flight sequence. Sixty contiguous coronal slices (thickness, 0.3 mm) were acquired with a field of view of 37×37 mm and a matrix size of 256×256 (TR=25 ms, TE=5 ms, TA=25.5 min, flip angle=90°, 3 averages), and a moving saturation slice of 20-mm thickness at the cranial side of the measured slice for suppression of venous signal. Three-dimensional reconstruction (maximum intensity projection) was performed with use of the Image Processing Tool of the Paravision 2.1 software (Pharmascan) after interpolation to isotropic voxel size.

**Clinical Evaluation**

Neurological evaluation was performed 3 hours and 24 hours after induction of ischemia and scored on a 7-point scale: 0, no neurological deficit; 1, failure to extend left forepaw fully; 2, incomplete circling to the left; 3, constant circling to the left; 4, falling to the left; 5, no spontaneous walking with depressed level of consciousness; and 6, death.

**MRI**

After clinical evaluation at 24 hours, the animals were placed in an MRI-scanner (Bruker PharmaScan 7.0T, 16 cm). Respiratory rate was monitored during the imaging protocol. Isoflurane concentration was varied between 2.0% and 3.0% to keep the respiratory rate between 60 and 80/min. Temperature was maintained at 37°C.

The linear polarized volume resonator (diameter, 60 mm) was tuned and matched manually.

The MRI tomography machine operates at 300.51 MHz for 1H imaging and is equipped with a 300-mT/m self-shielding gradient system. Localizer images were acquired using a spin-echo sequence. RARE sequences (20 contiguous slices, 1-mm thickness, TR=2500 ms, TE=41.8 ms) were used to verify symmetric positioning and were repeated after correction of slice angulation, if necessary.

**T2-Weighted Imaging**

High-resolution multislice proton-weighted and T2-weighted double-contrast spin-echo imaging was used to map lesion and hemispheric volumes. Sixteen contiguous coronal slices (thickness, 2 mm) were acquired with a field of view of 37×37 mm and a matrix size of 512×256 [repetition time (TR)=3000 ms, echo time 1 (TE1)=27 ms, echo time 2 (TE2)=72 ms, imaging time=25.5 min, 2 averages].

Computer-aided planimetric assessment of the lesion and hemispheric volumes were performed using image analysis software (Image J 1.25s; National Institutes of Health). After adjustment of contrast, the contours of the hemispheres were traced manually on each slice. The position of the midline was determined using neuroanatomic landmarks as described previously.7 Lesion volumes were determined by computer-aided manual tracing of the hyperintense lesions and corrected for the space-occupying effect of brain edema as described previously using the following equation:

\[
\%\text{HLV} = \frac{\text{HCc} - \text{HVc} + \text{LVu}}{\text{HVc}} \times 100
\]

where \(\%\text{HLV}\) indicates edema–corrected lesion volume (in percent of the hemispheric volume); \(\text{HCc}\) and \(\text{HVc}\) indicate contralateral and ipsilateral hemispheric volume; and \(\text{LVu}\) indicates uncorrected lesion volume.

**Figure 1.** Time course of body temperature. Body temperature increased in the permanent suture MCAO group only (group II). In groups I and III, temperature remained within the normal range.
where %HLV indicates edema–corrected lesion volume (in percent of the hemispheric volume); LV indicates direct lesion volume; and HV_i indicates ipsilateral hemispheric volume.

Hypothalamic damage was determined from the TTC-stained slices as described previously. Determination of infarct size and hypothalamic injury was performed by an experienced investigator, blinded to group assignment and clinical assessment.

**Statistics**
Data are presented as mean±SD. Parametric and nonparametric data were compared using Student t test or Mann–Whitney U test. Model failure rate was compared among the groups using Pearson χ² test. P<0.05 was considered statistically significant.

**Results**

**Physiological Parameters**
Blood pressure, pO₂, pCO₂, pH, and glucose measured 10 and 100 minutes after MCAO were within the physiological range and were not significantly different from baseline values or among the groups (P<0.05; data not shown).

**Clinical Findings**
Body weight and clinical scores at 3 hours and at 24 hours after MCAO did not differ among the groups (P>0.05). Body temperature remained stable in groups I and III but increased significantly in group II (P<0.01, compared with group I and III) (Figure 1, Table 1).

**MRI Findings**
Occlusion of the MCA main stem could clearly be demonstrated on MRI (Figure 2). Mean ischemic lesion size was significantly smaller in the reperfusion group compared with groups II (P<0.01) and III (P<0.05). Animals subjected to macrosphere MCAO had smaller lesion volumes compared with animals subjected to permanent suture MCAO (group II). This difference, however, was not statistically significant (P>0.05).

**TTC Findings**
Edema–corrected lesion volumes correlated well between MRI and TTC staining (group I, r=0.95; group II, r=0.69; group III, r=0.87).

**TABLE 1. Clinical Findings, Lesion Volume, and Model Failure Rate**

<table>
<thead>
<tr>
<th>Group</th>
<th>Score at 3 h</th>
<th>Score at 24 h</th>
<th>Temperature at 3 h</th>
<th>Temperature at 24 h</th>
<th>%HLV at 24 h</th>
<th>Model Failure Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: suture (reperfusion)</td>
<td>1.9±1.2</td>
<td>2.1±1.0</td>
<td>37.4°C±0.7</td>
<td>37.2°C±0.5</td>
<td>14.6%±12.4</td>
<td>1/11 rats (9%)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>1 (1–4)</td>
<td>2.5 (1–5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II: suture (permanent)</td>
<td>2.1±0.9</td>
<td>1.9±0.9</td>
<td>38.8°C±0.5</td>
<td>38.0°C±0.5</td>
<td>35.2%±11.4</td>
<td>4/14 rats (29%)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III: macrosphere (permanent)</td>
<td>2.0±1.1</td>
<td>2.0±1.1</td>
<td>37.4°C±0.4</td>
<td>37.3°C±0.5</td>
<td>21.3%±17.2</td>
<td>2/12 rats (17%)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Clinical scores did not differ among the groups or between the time points (P>0.05).
Compared to groups I and III, body temperature was significantly higher in group II 3 hours (P<0.01) and 24 hours (P<0.01) after MCAO.
Lesion volume was significantly smaller in the reperfusion group, compared to groups II and III.
The difference between groups II and III was not significant (P>0.05).
Model failure rate did not differ significantly among the 3 models (P>0.05).

%HLV indicates percent hemispheric lesion volume.

**Figure 2.** MCAO techniques. Top, Suture technique. After permanent MCAO, MRA (left) indicates occlusion of the ICA and MCA. The suture furthermore occludes the HA and the AChA that originate from the ICA (middle). Brain infarction in the territory of the MCA, AChA and HTA is clearly visible on T2-weighted imaging after 24 hours (right). Bottom, Macrosphere technique. After permanent MCAO, MRA (left) indicates occlusion of the MCA, but not of the proximal part of the intracranial portion of the ICA. The spheres block blood flow to the MCA and to the AChA, but not to the HA. This leads to brain infarction in the MCA and AChA territory, but not in the hypothalamic region (MRI, right). ACA indicates anterior cerebral artery; BA, basilar artery; AChA, anterior chorid artery.
Model Failure Rate

Seven animals had to be excluded because of the prespecified exclusion criteria and were replaced (Table 2):

Two animals (1 each in group II and III) did not show neurological deficits 3 hours after MCAO. TTC staining revealed no ischemic lesion after 24 hours. Inspection of the basal cerebral arteries revealed an anatomic variant in 1 animal (group III) with a doubled MCA main stem.

A patent MCA main stem was detected on MRA in 2 animals subjected to permanent MCA suture occlusion. In these animals, small subcortical lesions were detected on MRI, suggestive of an infarction in the territory of the anterior choroid artery (AChA) (Figure 3). Both animals showed neurological deficits.

One animal in group III displayed additional ischemic lesion in the territory of the HA, resulting in hyperthermia. SAH was detected in 2 animals on postmortem examination (1 each in group I and II) (Figure 4).

Table 2: Excluded Animals

<table>
<thead>
<tr>
<th>Score at 3 h</th>
<th>MRA</th>
<th>T2-weighted MRI</th>
<th>Postmortem Inspection</th>
<th>Temperature</th>
<th>TTC Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (suture, reperfusion)</td>
<td>1 MCA patent</td>
<td>MCA territory lesion</td>
<td>Subarachnoid hemorrhage*</td>
<td>Normal</td>
<td>MCA territory lesion</td>
</tr>
<tr>
<td>Group II (suture, permanent occlusion)</td>
<td>2 MCA patent*</td>
<td>Small lesion, suggestive of AChA and hypothalamic artery infarction</td>
<td>Normal</td>
<td>Hyperthermia</td>
<td>Small lesion, suggestive of AChA and hypothalamic artery infarction</td>
</tr>
<tr>
<td></td>
<td>1 MCA patent*</td>
<td>Small lesion, suggestive of AChA and hypothalamic artery infarction</td>
<td>Normal</td>
<td>Hyperthermia</td>
<td>Small lesion, suggestive of AChA and hypothalamic artery infarction</td>
</tr>
<tr>
<td></td>
<td>3 MCA occluded</td>
<td>MCA territory lesion</td>
<td>Subarachnoid hemorrhage*</td>
<td>Normal</td>
<td>MCA territory lesion</td>
</tr>
<tr>
<td></td>
<td>0* MCA patent</td>
<td>No ischemic lesion</td>
<td>Normal</td>
<td>Normal</td>
<td>No ischemic lesion</td>
</tr>
<tr>
<td>Group III (macrosphere, permanent occlusion)</td>
<td>3 MCA occluded</td>
<td>Lesion in the territory of the MCA and the hypothalamic artery*</td>
<td>Spheres occluded proximal ICA and MCA orifice</td>
<td>Hyperthermia</td>
<td>Lesion in the territory of the MCA and the hypothalamic artery*</td>
</tr>
<tr>
<td></td>
<td>0* MCA patent</td>
<td>No ischemic lesion</td>
<td>Doubled MCA main stem; spheres occluded only 1 main stem</td>
<td>Normal</td>
<td>No ischemic lesion</td>
</tr>
</tbody>
</table>

Seven animals (18.9%) were excluded. Clinical findings, MRI, and MRA data are presented for the individual animals. MCA indicates middle cerebral artery; ICA, internal carotid artery.

*Reason for exclusion.

Discussion

In the present study, 3 different endovascular MCAO techniques were compared. In each model, complications and side effects occurred that need to be considered to avoid misinterpretation of the results.

Verification of MCAO

Endovascular models can fail to occlude the MCA main stem.1,10 These animals need to be identified and excluded from studies. In the present study, we used a combination of early clinical evaluation and time-of-flight MRA to confirm MCAO.

MRA

Time-of-flight MRA allows the assessment of the intracranial vascular status in rodents noninvasively without using contrast agents. MRA thus is noninvasive and can easily be repeated (ie, to document sufficient MCAO and reperfusion).9,10

In the present study, MRA provided easily visualized imaging of the circle of Willis. Occlusion of the ipsilateral MCA was easy to verify. As expected, the ipsilateral MCA was patent in all rats in group I, indicating sufficient reperfusion after withdrawal of the suture. MCAO was confirmed in all animals in group III and in all but 2 rats in group II.

Figure 3. Model failure (permanent suture MCAO). Inappropriate insertion depths of the suture caused an occlusion of the AChA and the HA, but not of the MCA (left/middle). T2-weighted MRI revealed a small subcortical lesion, suggestive of AChA and HTA territory infarction (right). The animal showed moderate to severe neurological deficits and increased body temperature after 3 hours and 24 hours.
placement of the laser Doppler probes requires insufficient MCAO and reperfusion. In contrast to MRA, both methods provide (semi-)quantitative information about cerebral perfusion. These methods, however, have some disadvantages. Placement of the laser Doppler probes requires prolonged anesthesia and trepanation of the skull, leading to various side effects, such as reduction of intracranial pressure and local brain temperature, imaging artifacts, and others, and thus might confound experimental stroke studies. Perfusion-weighted imaging can lead to false-positive results caused by occlusion of the ipsilateral ICA, which can reduce perfusion in the MCA territory. Furthermore, perfusion imaging might indicate a reduced local cerebral perfusion in case of AChA occlusion, despite the MCA being patent, and thus lead to false-positive results (Figure 3).

Postmortem inspection of the circle of Willis theoretically allows one to determine the correct position of the occluder within the basal cerebral arteries. This procedure, however, might not be reliable for the suture model (and thus has not been performed in the present study), because dislocation of the suture can occur during the process of decapitation and removal of the brain. Using the macrosphere model, in contrast, the site of intracranial vessel occlusion can be determined reliably by visual inspection of the circle of Willis, because the spheres are lodged tightly within the arteries and cannot be dislocated by manipulation during the process of decapitation and brain removal. In the present study, we found a perfect agreement between MRA findings and verification of MCA main stem occlusion based on visual inspection of the circle of Willis in the macrosphere group.

Subarachnoid Hemorrhage
SAH results from perforation of the intracranial portion of the ICA or of the anterior cerebral artery and represents a typical complication of the suture MCAO model. This complication was confirmed by MRI (Figure 4) and postmortem examination in 1 out of 11 animals in group I (9%) and in 1 out of 14 rats in group II (7%). SAH can lead to serious side effects such as vasospasm and intracerebral hemorrhage, and thus can confound the results of experimental stroke studies. Previous reports indicated that laser Doppler-guided placement of the suture might reduce the incidence of SAH. In the present study and in previously published experiments, no case of SAH was documented in animals subjected to macrosphere MCAO. Thus SAH may not occur in this model.

Hypothalamic Infarction and Hyperthermia
Previous studies indicated that hyperthermia is accompanied by an ischemic lesion of the hypothalamic region. The pathological increase in temperature after permanent suture MCAO (group II) can be explained by obstruction of the HA that originates from the distal portion of the ICA, because the suture occludes the entire carotid artery from the bifurcation to the origin of the MCA (Figure 2). Hyperthermia did not occur if the suture was removed within the first 90 minutes after MCAO (group I). Hypothalamic damage and subsequent hyperthermia are important complications in experimental stroke research. This condition has been shown to nullify the neuroprotective effect of the NMDA receptor antagonist MK-801 in permanent MCAO. In the present study, hypothalamic damage and pathological hyperthermia were constant findings in permanent MCAO using the suture technique (group II). Reperfu-
sion at 90 minutes (group I) and use of the macrosphere technique for permanent occlusion (group III) efficiently avoided this side effect (Figures 1 and 2). Hypothalamic damage was detected in only 1 animal in group III which has been excluded.

**Conclusion**

Failures and side effects occur frequently in endovascular stroke models. In the present study, insufficient occlusion of the MCA was documented in the suture model and in the macrosphere technique. These animals cannot be identified reliably on the basis of clinical examination, because some rats display hemiparesis caused by ischemic lesions in the territory of the AChA, although the MCA was not occluded. Therefore, additional methods to verify MCAO are warranted.

The present study proved MRA to be a reliable and noninvasive method for the verification of MCAO. Based on clinical findings alone, only 2 out of 4 animals with insufficient MCAO were identified. The 2 remaining animals would have not been detected without the use of MRA.

SAH was detected in 2 out of 25 animals subjected to suture MCAO. This side effect did not occur in the macrosphere model and appears to be a specific complication of the suture techniques.

Hypothalamic infarction and pathological hyperthermia could be verified in all animals subjected to permanent suture MCAO. This side effect can be avoided by withdrawal of the suture after 90 minutes or by using the macrosphere technique for permanent MCAO.

**References**

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