A New Method to Improve In-Bore Middle Cerebral Artery Occlusion in Rats
Demonstration With Diffusion- and Perfusion-Weighted Imaging

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Background and Purpose—In-bore middle cerebral artery occlusion (MCAO) enables investigators to acquire preischemic MRI data and to image ischemic changes immediately after occlusion. We have developed a highly successful in-bore MCAO method. This study describes the methods and pertinent techniques.

Methods—Sixty-seven Sprague-Dawley rats were subjected to temporary (n=36) or permanent (n=31) MCAO. The occluding device consisted of a supporting tubing, a driving line, and a silicone-coated 4-0 nylon suture occluder. Outside the magnet, the occluder was positioned in the carotid canal. MCAO was achieved in the magnet bore by remotely advancing the driving line until resistance was felt. Diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI) were acquired before and immediately after occlusion and were used to document the presence of MCAO.

Results—Fifty-nine (88.1%) rats were successfully occluded, demonstrating hyperintensity on DWI, perfusion deficits on PWI, and no subarachnoid hemorrhage at postmortem examination. The average values of the apparent diffusion coefficient in both the frontoparietal cortex and the lateral caudoputamen significantly decreased as early as 3 minutes after the onset of ischemia. The failures included preocclusion damage (1/67), sliding out of the occluder during occlusion (1/67), no occlusion (2/67), and arterial perforation (4/67).

Conclusions—Our in-bore MCAO method is easily performed and is as successful as MCAO induced outside the magnet. (Stroke. 1998;29:1715-1720.)

Key Words: cerebral ischemia, focal ■ magnetic resonance imaging ■ middle cerebral artery occlusion ■ rats

Diffusion-weighted imaging is sensitive for the early detection of focal brain ischemia both in animals and in humans. The acutely ischemic region appears hyperintense on DWI, and these ischemic changes can be quantified by measuring the ADC of water. When animal stroke models are combined with this novel imaging technique, the pathophysiological changes of ischemia can be extensively evaluated. Usually, focal cerebral ischemia is induced outside the magnet bore, preventing investigators from acquiring preischemic images for later pixel-by-pixel comparisons and from studying the earliest imaging changes of ischemia. Recently, the development of an MCAO method inside the magnet bore, so called “in-bore occlusion,” has made it possible to acquire preischemic MRI data and to visualize ischemic changes on DWI immediately after the onset of ischemia. However, the methods are not highly successful and the techniques required are of limited availability. We have developed a highly successful in-bore MCAO method. In this article, we summarize the application of this method in both temporary and permanent MCAO and discuss the relevant techniques.

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Materials and Methods

Animal Preparation
This study was approved by the Animal Research Committee of the University of Massachusetts Medical School (Docket #643). Sixty-seven male Sprague-Dawley rats weighing 300 to 340 g were anesthetized intraperitoneally with 400 mg/kg chloral hydrate. PE-50 polyethylene tubing was inserted into the left femoral artery for monitoring blood pressure and for measuring pH, PaCO₂, and PaO₂ before and after MCAO. Another PE-50 polyethylene tubing was inserted into the left femoral artery for injecting gadopentate dimeglumine (Magnavist, Berlex Laboratories). Temperature was continuously monitored with a rectal probe and maintained at 37°C with a thermostatically controlled heating lamp.

In-Bore MCAO Method
In a pilot study, 14 male Sprague-Dawley rats weighing 280 to 340 g were used to explore the most suitable in-bore MCAO method, including the choice of an appropriate suture occluder, driving line,
and rat's body weight. After the method was optimized, 36 of 67 rats were subjected to permanent MCAO. The in-bore MCAO was induced with an occluding device, composed of a 110-cm supporting tubing (ID=1.14 mm, OD=1.65 mm), 0.5 cm connecting tubing (ID=0.86 mm, OD=1.27 mm), 1.5-cm intra-arterial tubing (ID=0.35 mm, OD=0.75 mm), 115-cm driving line (30 lb monofilament line, MAXIMA MFG Co), and 3-cm occluder connected to the end of the driving line with a 2-cm PE-50 polyethylene tubing and glue (Figure 1). The occluder was a 4.0-monofilament nylon suture (4.0 Ethilon, Ethicon Inc) with its tip rounded by flame heating and uniformly coated with silicone.12 The right CCA, ECA, and ICA were exposed through a ventral midline incision in the neck. The proximal portions of the CCA and ECA were ligated tightly with a 6-0 suture and transected. The ICA was further dissected distally until it passed through the cranial base. The right pterygopalatine artery was ligated and transected. The ICA was further dissected distally until it passed above the bifurcation, and fixed by double ligation (Figure 2). The proximal portions of the CCA and ECA were ligated tightly with a 3-0 suture. The right occipital artery was ligated with a 6-0 suture. The proximal portions of the CCA and ECA were ligated tightly with a 6-0 suture and transected. The ICA was further dissected distally until it passed through the cranial base. The right pterygopalatine artery was ligated with a 6-0 suture. The animal was then fixed on a head holder with ear-bars and a tooth-bar. The supporting tubing was attached to the wall of the chest and abdomen and the proximal portion of the stenomastoid muscle by 5 stitches with a 0 suture. Then the intra-arterial tubing was inserted through an arteriotomy of the CCA 3 mm below the carotid bifurcation, advanced into the ICA 2 mm above the bifurcation, and fixed by double ligation (Figure 2). The driving line was gently advanced until the occluder passed through the carotid canal and the end part of the driving line was carefully protected. Then the rat's head was inserted into a H “birdcage” imaging coil, and the holder was placed into the magnet bore with great care. Inside the magnet, anesthesia was maintained with 1.0% isoflurane delivered in air at 1 L/min. Temperature was continuously monitored using a rectal probe and maintained at 37°C by means of a thermostatically regulated airflow system. MCAO was achieved in the magnet bore by remotely advancing the end of the driving line until resistance was felt, indicating that the occluder had entered the ACA and thus had blocked the blood flow from the ACA and the PCA to the MCA. Further advancement was attempted if no MCAO was documented by DWI and PWI. The depth of the occluder above the carotid bifurcation was acquired by measuring the difference of the end distance of the driving line outside the supporting tubing when the tip of the occluder was just above the bifurcation and when occlusion was accomplished. For temporary MCAO, the animals were reperfused by withdrawing the driving line approximately 10 mm. For permanent MCAO, the occluder was carefully fixed to the ICA with a 3-0 suture after the MRI protocol.

MRI Measurements

MRI data were acquired with a GE CSI-II 2.0T/45 cm imaging spectrometer (GE NMR Instruments) operating at 85.56 MHz for the 1H and equipped with ±20 G/cm self-shielding gradients. Eight contiguous coronal slices of 2 mm in thickness were acquired with a field of view of 25.6×25.6 mm and a matrix size of 64×64 (TR=2 [temporal MCAO] or 5 seconds [permanent MCAO], TE=57 milliseconds, EPI data acquisition time=65 milliseconds, NEX=6 [permanent MCAO] or 2 [permanent MCAO]). Five b values (67 to 1671 s/mm², temporary MCAO) or 9 b values (119 to 2409 s/mm², permanent MCAO) were used to measure ADC of water along each of the 3 orthogonal gradient axes. The ADCw value for each pixel before and after MCAO was compared. The in vivo lesion volume of regions with a 29% reduction of the ADCw value was highly correlated with the postmortem infarct volume (F. Li, unpublished data, 1997). Thus, the pixels with a >29% ADCw were used to define abnormal. The number of abnormal pixels divided by the total number of pixels in the ischemic hemisphere from slice 2 to slice 7 that matched the 6 mm brain slices at postmortem TTC staining, was used to calculate %HLV.

\[ T2^* \text{ weighted EPI was used to perform PWI. Four contiguous coronal slices with a 2-mm thickness were acquired with a field of view of 25.6×25.6 mm and a matrix size of 64×64 (TR=500 milliseconds, TE=38 milliseconds, EPI data acquisition time=65 milliseconds, NEX=1). A total of 25 images were obtained for each slice. A bolus injection of 0.15 mL of gadopentate dimeglumine was administered after the seventh imaging acquisition. The relative hyperintensity in the ipsilateral MCA territory, compared with the reduction of signal intensity in normally perfused tissue due to T2* shortening effects caused by the contrast agent, was defined as a perfusion deficit.}^{15} \text{ CBFI maps were acquired from the PWI data using a previously described method.}^{14} \text{ Abnormal pixels were defined as those with CBFI values that fell 2 SDs below the mean of the} \]
CBF values in the normal hemisphere. The number of abnormal pixels divided by the total number of pixels in the ischemic hemisphere was yield %HLV. DWI followed by PWI was acquired before and immediately after MCAO in both groups, immediately and every 30 minutes for a total of 90 minutes after reperfusion in the temporary MCAO group, and every 30 minutes for a total of 270 minutes after MCAO in the permanent MCAO group. Two regions of interest, one in the frontoparietal cortex and the other in the lateral caudoputamen, were chosen to quantify the ADC av before and immediately after MCAO, because these 2 areas are typically involved with the ischemic injury in this suture occlusion model.15–16 Postmortem examination was performed to verify subarachnoid hemorrhage in all rats. Twenty-four hours after MCAO, the rats in the permanent MCAO group had their infarct volumes calculated using a TTC-derived %HIV. The ADC av -derived %HLV grew significantly, from 11.2% immediately after MCAO. Twenty of the 27 successfully occluded rats in the permanent MCAO group survived for 24 hours after MCAO and were used to evaluate the evolution of ischemic lesion volumes and final infarct volumes. The ADC av -derived %HLV grew significantly, from 8.7±6.7% at 5 minutes to 17.6±11.6% at 30 minutes after MCAO (paired t test, P<0.003), almost maximized (28.5±10.0%) at 150 minutes after MCAO, and was 29.3±9.7% at 270 minutes. The TTC-derived %HIV at 24 hours after MCAO was 28.8±5.8%. Figure 3 shows that the ADC av -derived %HLV at 270 minutes after MCAO was highly correlated with the TTC-derived %HIV (r=0.90, P<0.0001).

**Results**

The physiological parameters such as body temperature, arterial blood pressure, pH, Paco2, and Pao2 before and after MCAO were not significantly different (paired t test, data not shown), although there was a trend toward a decrease in Paco2 and an increase in Pao2 after MCAO.

Normal DWI and PWI were observed in 66 rats before MCAO, and a preocclusion perfusion deficit occurred in 1 rat. Hyperintensity on DWI and perfusion deficits on PWI in the occluded MCA territory, mainly involving the frontoparietal cortex and underlying caudoputamen, were present in 63 of these 66 rats almost immediately or shortly after occlusion. The occluder spontaneously slid out during occlusion in 1 rat and could not be advanced intracranially in 2 rats, giving rise to no occlusion. At postmortem examination, subarachnoid hemorrhage was observed in 4 of the 63 rats that developed hyperintensity on DWI and perfusion deficits on PWI. Therefore, 59 (32 in the temporary MCAO group and 27 in the permanent MCAO group) of 67 (88.1%) rats were successfully occluded. Successful occlusion was accomplished during the first attempt in 45 rats, the second attempt in 8 rats, the third attempt in 5 rats, and the fourth attempt in 1 rat. The mean depth of the occluder above the bifurcation was 17.5±0.5 mm (range, 17 to 19.5 mm). The depth that the occluder had to be advanced in rats with a body weight over 320 g was significantly greater than that in rats with a body weight of 320 g or less (17.9±0.5 mm versus 17.2±0.2 mm, unpaired t test, P<0.001).

The Table shows that the ADC av values in the frontoparietal cortex and lateral caudoputamen significantly decreased (paired t test, P<0.001) immediately after MCAO in both the temporary and permanent MCAO groups, compared with the preischemic ADC av values. The ADC av-derived %HLV was 5.1±6.2%, and the %HLV on perfusion imaging was 28.8±11.2% immediately after MCAO. Twenty of the 27 successfully occluded rats in the permanent MCAO group survived for 24 hours after MCAO and were used to evaluate the evolution of ischemic lesion volumes and final infarct volumes. The ADC av-derived %HLV grew significantly, from 8.7±6.7% at 5 minutes to 17.6±11.6% at 30 minutes after MCAO (paired t test, P<0.003), almost maximized (28.5±10.0%) at 150 minutes after MCAO, and was 29.3±9.7% at 270 minutes. The TTC-derived %HIV at 24 hours after MCAO was 28.8±5.8%. Figure 3 shows that the ADC av-derived %HLV at 270 minutes after MCAO was highly correlated with the TTC-derived %HIV (r=0.90, P<0.0001).

**Discussion**

DWI and PWI can rapidly and reliably document the early changes of cerebral ischemia.1–3 By using DWI and PWI to

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**Preocclusion and Postocclusion ADC av Values (×10⁻⁵ mm²/s) of the Frontoparietal Cortex and Caudoputamen in Both Temporary and Permanent MCAO Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Frontoparietal Cortex</th>
<th>Lateral Caudoputamen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ADC av)pre</td>
<td>(ADC av)post</td>
</tr>
<tr>
<td>Temporary MCAO</td>
<td>68.8±4.3</td>
<td>59.2±7.9*</td>
</tr>
<tr>
<td>Permanent MCAO</td>
<td>65.7±2.4</td>
<td>51.0±7.2*</td>
</tr>
</tbody>
</table>

(ADC av)pre indicates preocclusion ADC av value; (ADC av)post, postocclusion ADC av value (3 and 5 minutes after MCAO in temporary and permanent occlusion groups, respectively).

*P<0.001 (ADC av)post vs (ADC av)pre.

**Statistical Analysis**

Data are presented as mean±SD. Statistical analyses were performed using a paired or unpaired t test. Linear regression was used to correlate the ADC av-derived %HLV with TTC-derived %HIV. A 2-tailed value of P<0.05 was considered significant.

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**Figure 3.** The correlation between ADC av-derived %HLV at 270 minutes after MCAO and TTC-derived %HIV at 24 hours after MCAO (n=20). A significant correlation is acquired (r=0.90, P<0.0001).
monitor the presence of ischemia, our study demonstrated that our new in-bore MCAO method is highly successful and reliable as well as straightforward in both the temporary and permanent occlusion of rats.

The in-bore occlusion method was developed by modifying the suture MCAO model.12-11 MCAO was remotely induced by further advancing the occluder 10 mm,2 10 to 11 mm,10 or 9 to 12 mm.11 Although the distance between the tympanic bulla and the MCA was found to be consistent (9 mm),11 the variability of the ACA in diameter could lead to a high failure rate (25.6% to 30.8%)10,11 of MCAO if the occlusion depends on the exact depth. These failures included perforation of the ICA or ACA and no occlusion. Kohno et al10 demonstrated that simultaneous EEG monitoring can reduce the arterial perforation rate from 30% to 5%, but the manipulation of EEG electrodes is difficult and the animals may incur additional injury. Previous studies did not discuss the cause of failures and related technical details. We believe a proper type of occluder is important. The originally described silicone-coated 4-0 suture occluder was found to be most suitable for our in-bore MCAO, which has been demonstrated to produce reliable occlusion and reproducible lesion size.12 Also, the driving line is crucial to reduce the failure rate. Previously, arterial perforation occurred in 20.5% (8/39) of rats when a polyethylene catheter was used as a driving line,10 and no lesion developed in 23.1% (3/13) when 10 lb monofilament was used.11 We found that a 30-lb monofilament line is appropriate for the driving line. With this line, it was easy to advance the occluder and, more importantly, resistance could be felt when the occluder properly stopped in the ACA. Feeling resistance, a very important sign for proper MCAO, indicates that the tip of the occluder is tightly lodged in the ACA without arterial puncture and that the blood flow from the ACA to the MCA is maximally interrupted.18 Furthermore, the coated body of the occluder maximally prevents the blood flow from the PCA and perforating artery.17 Based on feeling this resistance, we successfully occluded 88.1% (59/67) of the rats, similar to the success rate (92%) of MCAO induced outside the magnet.15 Among the rats with successful occlusion, 76.3% (45/59) were successfully occluded on the first try. Lack of occlusion on the first try was most likely to be related to “false” resistance caused by clot formation within the occluding device and the occluder stopping proximally to the orifice of MCA. A further gentle advance resulted in successful occlusion with less risk of arterial perforation because of the soft and malleable coating we used.12,18 In this study, arterial perforation occurred in only 6% (4/67), and no occlusion occurred in only 3% (2/67) of the rats. Other problems were preocclusion damage (1/67) and the sliding out of the occluder during occlusion (1/67). The following procedures might be used to minimize these failures. First, moistening the occluding device with heparinized saline is more likely to easily advance the driving line, because blood clot formation within the occluding device can give rise to false resistance or even prevent the driving line from advancing. Second, after the tip of the occluder is positioned at the carotid canal, the driving line should be secured, because advancing or withdrawing the driving line accidentally will result in preocclu-

sion damage or no occlusion. Once the occluder slides out from the cranial base, it is difficult to advance it within the magnet. Third, the driving line should be carefully and gently advanced during occlusion and stopped immediately when resistance is felt. Then the depth of the occluder should be checked. The feeling of resistance plus a depth of around 17 mm likely indicate successful occlusion. Finally, after occlusion has been induced, the driving line should be securely fixed during occlusion to avoid its sliding out of the occluder.

Our results showed that the depth of the occluder above the bifurcation was variable (17 to 19.5 mm) when a uniform occluder size was used in rats with a body weight of 300 to 340 g, consistent with a previous report.15 This variability is associated with the animal’s body weight and anomalies of the carotid bifurcation. In our pilot study, we found that in-bore MCAO was frequently unsuccessful in rats with a body weight of less than 300 g. We did not apply our in-bore MCAO method to any other strains of rats. Kohno et al10 previously tried the in-bore MCAO method in Fischer and Wistar rats, and found out that their method was suitable for Wistar rats but not for Fischer rats. Therefore, the suitability of our in-bore MCAO method to other rat strains needs further exploration.

Our results also demonstrated that a significant ADC drop occurred as early as 3 minutes after the onset of ischemia, consistent with the finding of Rother et al,11 and supported the hypothesis that the ADC decrease is associated with energy depletion and loss of ion homeostasis because the latter coincidentally occurs about 2 to 3 minutes after ischemia begins.20 The ischemic lesion size at very early time points after occlusion was quite variable, likely attributable to an inhomogeneous decline in ADC, then increased rapidly, and maximized approximately 2 to 3 hours after MCAO in this in-bore MCAO model. This is in agreement with a previous report using a permanent MCAO method induced outside the magnet bore.16 Because of its merits, in-bore MCAO has been used to investigate the temporal evolution of hyperintensity on DWI at very early times,9 the regional relationship between the signal intensity change of DWI, energy metabolism, and cerebral blood flow,21,22 and the early appearance of cortical spreading depression.11 Recently, Hoehn-Berlage et al23 evaluated the effectiveness of a neuroprotective drug with the in-bore MCAO method.

In conclusion, the advantages of our in-bore MCAO method are that resistance can be remotely felt when the occluder enters the proximal lumen of the ACA, it is easily performed, it can be applied to both temporary and permanent MCAO modeling, and it is as successful as MCAO induced outside the magnet. We anticipate that the in-bore MCAO method will be useful for investigating the pathophysiology of brain ischemia and for evaluating the efficacy of drug treatment.

Acknowledgments

This study was supported in part by the Harrington Neurological Research Fund. Dr Li was supported in part by an International Fellowship (F05 TWNS05272-01) from the Fogarty International Center of the National Institutes of Health. Part of this study was...
performed during the tenure of an established investigatorship from the American Heart Association (Dr Sotak).

References


Editorial Comment

Recent exploration of newer MRI techniques in stroke research has led to the use of diffusion-weighted imaging (DWI) to identify regions of tissue at risk and perfusion-weighted imaging (PWI) to define regions of perfusion deficit within hours of ischemic insult. Tong et al have demonstrated that the lesion volumes defined by DWI and PWI in patients at the hyperacute stage (<6.5 hours from onset) are highly correlated with stroke severity as defined by the NIH Stroke Scale 24 hours after the onset of ischemia. Moreover, it has been proposed by both Warach et al and Sorensen et al that when both DWI and PWI are used, it is possible to identify regions suggestive of penumbra in patients with acute stroke. However, before the newer MR techniques are validated and accepted in clinical practice, more studies are needed to define the pathophysiological correlates of DWI and PWI abnormalities in acute cerebral ischemia. Animal stroke models have been extensively used for this objective. A major limitation of the current stroke models is the need to move the animals in and out of the MR scanner. In-bore occlusion of the cerebral vessels, especially the middle cerebral artery (MCA), will allow immediate assessment of MR changes during the peri-ischemic period. It also makes comparison of MR parameters before and after ischemia easier and more reliable.

The preceding article by Li and colleagues describes an innovative approach to achieve in-bore MCA occlusion (MCAO) in rats. Kucharczyk et al demonstrated earlier that focal ischemia can be induced without moving the animals in and out of the magnet via an occlusion catheter in cats, which are substantially larger in size. The small animals used by Li et al present extra technical challenges to induce consistent MCAO. In the preceding article, the length of the occluding device was increased to 110 cm. This is particularly advantageous for those experiments conducted...
in a whole-body MR imager. However, with the increased length of the occlusion device, it is technically more difficult to obtain consistent and reproducible results. Results presented in this article provide preliminary findings suggesting that it is technically feasible to induce substantial and reproducible focal cerebral ischemia within the scanner in nearly 90% of the rats. In addition to the conventional TTC-derived lesion volume, a significant reduction of ADC values in the fronto-parietal cortex and lateral caudoputamen was observed immediately after the onset of ischemia; consistent with the expected ADC behaviors reported in the literature when focal ischemia was induced outside the magnet. While the ADC-derived lesions correlated with the TTC-derived infarct volumes in this study, it should be noted that the finding was based on a selected group of 20 rats with permanent MCAO, which is more likely to result in consistent focal cerebral ischemia. Because no comparison between in-bore and out-of-bore MCAO was made in the present study, it is difficult to fully realize the advantage of this new model over the conventional MCAO models. Notwithstanding, the ability to conduct in-bore MCAO in a whole-body scanner raises the hope that this new model may allow comparison of regions of DWI and PWI abnormalities on a pixel-by-pixel basis directly before and after the onset of ischemia without the requirement of coregistration. Using this method, there will be no time delay between the onset of ischemia and the MR acquisition. In conjunction with the high temporal resolution as well as the timely acquisition of MR data, the ability to detect rapid and more subtle DWI and PWI changes caused by ischemia may be greatly enhanced.

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Stroke. 1998;29:1715-1720
doi: 10.1161/01.STR.29.8.1715

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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