Comparison of Ischemic Lesion Evolution in Embolic Versus Mechanical Middle Cerebral Artery Occlusion in Sprague Dawley Rats Using Diffusion and Perfusion Imaging

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Background and Purpose—Differences among models in the temporal evolution of ischemia after middle cerebral artery occlusion (MCAO) in rats may considerably influence the results of experimental stroke research. Using diffusion and perfusion imaging, we compared the spatiotemporal evolution of ischemia in Sprague Dawley rats after permanent suture MCAO (sMCAO; n=8) and embolic MCAO (eMCAO; n=8).

Methods—Serial measurements of quantitative cerebral blood flow (CBF) and the apparent diffusion coefficient (ADC) were performed up to 180 minutes after MCAO. ADC and CBF values within 5 different brain regions were analyzed. ADC and CBF lesion volumes were calculated by using previously established viability thresholds and correlated with infarct volume defined by 2,3,5-triphenyltetrazolium chloride staining 24 hours after MCAO.

Results—Compared with sMCAO animals, the threshold-derived CBF lesion volume was significantly larger in eMCAO at all time points (P<0.01), remained relatively constant over time, and was highly correlated with the 2,3,5-triphenyltetrazolium chloride–defined infarct size. The ADC lesion volume did not differ between models at any time point. A diffusion/perfusion mismatch was present significantly longer in eMCAO animals (P<0.05), and these rats demonstrated larger absolute mismatch volumes that were statistically significant at 30, 60, and 90 minutes (P<0.05). In both models, CBF and ADC declines were highly correlated.

Conclusion—This study demonstrated substantial differences in acute ischemic lesion evolution between the eMCAO and sMCAO models. (Stroke. 2006;37:1283-1287.)

Key Words: animal models ■ cerebral ischemia, focal ■ magnetic resonance imaging, diffusion-weighted ■ magnetic resonance imaging, perfusion-weighted ■ stroke

The primary goal of acute stroke therapy is to prevent the ischemic penumbra from proceeding toward infarction, that is, to reduce ultimate infarct size translating into improved neurological and functional outcome.1 Identifying potentially reversible ischemic tissue with diffusion–perfusion MRI might, therefore, help to extend the therapeutic time window for effective reperfusion therapy.2

The suture middle cerebral artery occlusion (MCAO) model in the rat is an indispensable tool for unraveling the complex pathophysiology of permanent and transient focal cerebral ischemia.3 However, it does not reliably reproduce the inhomogeneous vascular findings at the microvascular and macrovascular level in patients experiencing acute territorial stroke, ranging from early recanalization and delayed or drug-induced recanalization to permanent vessel occlusion.4 Furthermore, the therapeutic window for certain therapeutic options may be substantially shorter in this model than in humans.2,5 Because the underlying pathophysiology of brain damage is likewise heterogeneous, embolic MCAO (eMCAO) is of increasing interest to study thrombolytic and neuroprotective drugs because it more closely simulates the pathophysiology of human MCAO with different degrees of inadvertent reperfusion,6,7 the possibility to apply thrombolytic therapy,7–9 and a potential time window for successful intervention >3 hours.9,10

Diffusion and perfusion imaging provides a simple and feasible means to identify ischemic tissue at risk for infarction beyond the established 3-hour time window.2 Therefore, these techniques have been used extensively to investigate acute lesion evolution in both animals and humans and aid decision-making in the clinical setting.2,11 The present study used diffusion and perfusion imaging to investigate potential differences in the spatiotemporal evolution of ischemia after permanent suture MCAO (sMCAO) and eMCAO in Sprague Dawley rats that may...
help explain reported differences in the time window for intervention in these models.

Materials and Methods

Animal Preparation
Male Sprague Dawley (n = 16) rats (Taconic Farms; Hudson, NY) weighing 289 ± 11 g were anesthetized with isoflurane (5% for induction, 2% for surgery, and 1.2% for maintenance) in room air. PE-50 polyethylene tubing was inserted into the femoral artery for monitoring of mean arterial blood pressure and for obtaining blood samples to measure blood gases (pH, PaO2, and PaCO2) and plasma glucose at baseline, 90, and 180 minutes after MCAO. Body temperature was monitored continuously with a rectal probe and maintained at 37.0 ± 0.5°C with a thermostatically controlled heating lamp. Permanent sMCAO (model 1) was produced in 8 animals as described previously.5 For eMCAO (model 2), 1 red blood clot (diameter 0.35 mm; length 36 mm) was injected into the internal carotid artery of 8 animals over ~1 s at the bifurcation of the pterygopalatine and internal carotid arteries as described previously.8

Embolus Preparation
Embolus preparation for embolus preparation used herein was modified from a protocol originally described by Toomey et al. Whole blood (200 μL) was withdrawn from the rat 24 hours before surgery into an Eppendorf tube. The blood was promptly mixed with 1.0 National Institutes of Health (NIH) unit (10 μL) of human thrombin and 4.5 μL of 1 mol/L CaCl2 for a final CaCl2 concentration of 20 mmol/L. Within 5 s, a small portion of this mixture was drawn into an 30.0-cm length of polyethylene catheter (PE-50) and allowed to clot at 37°C for 2 hours. At the end of this period, the clot was extruded from the catheter into a saline-filled Petri dish and stored at 4°C for 22 hours. Before embolism, a 5- to 10-cm section of clot was placed into a separate Petri dish containing deionized water, incubated for 5 minutes at room temperature, thereafter transferred into a solution of isotonic saline, and dissected into a single 36-mm section. This section (clot) was collected into a PE-10 catheter in a volume of 50 μL of saline. The interval between this final step and embolization was <5 minutes.

MRI Measurements
MRI experiments were performed on a 4.7 T/40 cm horizontal magnet equipped with a Biospec Bruker console, and a 20 G/cm gradient insert (ID 12 cm; 120-μs rise time). A surface coil (ID 2.3 cm) was used for brain imaging and an actively decoupled neck coil for perfusion labeling.4 Animals were imaged at 30, 60, 90, 120, 150, and 180 minutes after MCAO. A complete imaging block lasted 10 minutes. The apparent diffusion coefficient (ADC) of water and basal cerebral blood flow (CBF) were recorded. Three ADC maps were separately acquired with diffusion-sensitive gradients applied along the x, y, or z direction. Single-shot, echo-planar images were acquired over 2.5 minutes with matrix=64x64, spectral width=200 kHz, repetition time = t s (90° flip angle), echo time = 37.5 ms, b = 8 and 1300 s/mm2, Δt = 24 ms, δt = 4.75 ms, field of view = 2.56x2.56 cm, seven 1.5-mm-thick slices, and 16 averages. CBF measurements were made using the continuous arterial spin-labeling technique with single-shot, gradient-echo, echo-planar image acquisition. Paired images were acquired alternately: 1 with arterial spin labeling and the other without. Magnetic resonance (MR) parameters were as follows: matrix = 64x64, repetition time = 2 s (90° flip angle), echo time = 13.5 ms, field of view = 2.56x2.56 cm, seven 1.5-mm-thick slices, and 60 pairs of images.

Calculation of In Vivo Lesion Size
MRI measurements were analyzed using the imaging processing programs Matlab (Mathworks) and STIMULATE.4 Quantitative CBF and ADC maps were calculated as described previously.5,13 In vivo ADC and CBF lesion volumes were determined by using previously determined quantitative viability thresholds and methodology for previous studies using Sprague Dawley rats in our laboratory.5,13 These thresholds were used to identify all pixels with abnormal ADC or CBF characteristics on each of the 7 imaged slices at each time point in both the sMCAO and the eMCAO models. The corresponding ADC and CBF lesion volumes were then calculated by summing the abnormal area and multiplying by the slice thickness. The viability thresholds were 0.53x10^-3 mm/s for ADC and a reduction by 57% relative to the contralesional side for CBF.13

Region of Interest Analysis
Five regions of interest (ROIs; each 4×4 pixels) were defined manually at all imaging time points in the ipsilesional hemisphere on the ADC and initial CBF maps that were coregistered with the 2,3,5-triphenyltetrazolium chloride (TTC) results and compared with corresponding contralesional areas. The position of these ROIs remained constant over time and describe 5 possible tissue compartments: (1) benign oligemia defined by hyperperfusion above the critical perfusion threshold that correlated with no ADC or TTC lesions. Tissue hyperperfused below the threshold was subdivided in the following regions based on differences in ADC and TTC abnormality; (2) sustained reversal of initially reduced ADC and a TTC abnormality; (4) mismatch defined by delayed reduction of ADC and a TTC-defined infarct; and (5) core defined by reduced ADC below the critical threshold and a TTC abnormality. ROIs 1, 4, and 5 were chosen because they are frequently used to describe the 3 main compartments of ischemic stroke diagnosis and therapy in both animals and humans.11,14 In addition, ROIs 2 and 3 were selected because recent studies show their presence in human embolic stroke and suggest they may help define potential tissue fates within the diffusion/perfusion mismatch.15,16

Euthanization and Infarct Volume Analysis
After the last MR scan at 24 hours post-MCAO, animals’ brains were harvested and sectioned coronally into seven 1.5-mm-thick slices corresponding to the MR slices and stained with TTC for post mortem infarct volume calculation with edema correction.5 Animals dying prematurely between 16 and 24 hours after stroke onset were included in the data analysis.17

Statistical Analysis
Data are expressed as mean±SD. Infarct sizes, threshold-derived ADC and CBF lesion volumes, and mismatch volumes were compared among models by t test or Mann–Whitney rank sum test, as appropriate. Differences in ADC or CBF within ROIs and over time were statistically evaluated with ANOVA with post hoc Fisher least significance comparisons, as appropriate. Correlation analysis was conducted using the Spearman rank product. (Sigma-Stat 3.1; SPSS). P<0.05 was considered statistically significant.

Results

Mortality and Data Exclusion
Two (25%) and 3 (37.5%) animals died in the sMCAO and eMCAO model between 16 to 24 hours after MCAO, respectively. TTC-defined infarct volumetry was rapidly performed in those animals in addition to those surviving until elective euthanization at 24 hours. Because of technical reasons, no CBF values could be ascertained in 1 sMCAO animal.

Physiological Measurements
Basal physiological parameters did not differ significantly among models and they did not show any difference before and after sMCAO or eMCAO (data not shown). There was no significant difference in weight among the animals used for both models (data not shown).

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Temporal Evolution of ADC- and CBF-Derived Lesion Volumes and Correlation With TTC-Derived Infarct Volumes

The typical initial ADC and CBF lesion volumes for eMCAO and sMCAO rats are shown in Figure 1A and 1B, respectively. Although the ADC lesions were of equal size in both animals, the mismatch area was significantly larger in the eMCAO rat. Furthermore, there were intermodel differences in the location of the CBF lesion, which extended into the posterior cerebral artery (PCA) territory in eMCAO rats. Notably, MR angiography in a subset of eMCAO animals demonstrated occlusion of the ipsilesional PCA, which was not occluded in sMCAO rats (data not shown). Figure 2 summarizes the spatiotemporal evolution of threshold-derived ADC and CBF lesion volumes for both models. CBF-defined lesion volume was significantly larger in the eMCAO model relative to sMCAO rats at all time points (P<0.01) and remained relatively constant over time. In the sMCAO model, CBF-defined lesion volumes at each time point were highly correlated with the TTC-defined infarct volume at 24 hours after MCAO (correlation coefficient R ranging from 0.94 to 0.98; P<0.001 each). In the eMCAO model, the correlation between CBF-defined lesion volumes and TTC-defined infarct volume at 24 hours successively increased over time from 0.81 at 30 minutes (P<0.05) to 0.99 at 180 minutes (P<0.01). ADC lesion volume did not significantly differ between models at any time point (P>0.05).

Relative to the sMCAO model, eMCAO rats manifested a diffusion/perfusion mismatch with a significantly greater volume (at 30, 60, and 90 minutes; P<0.05) and duration (120 versus 60 minutes; P<0.05). In both experimental models, final corrected TTC-defined infarct volumes at 24 hours correlated significantly (P<0.001) with the threshold-defined CBF (eMCAO: R=0.996, P<0.001; sMCAO: R=0.948, P<0.001) and ADC (eMCAO: R=0.702, P<0.01; sMCAO: R=0.756, P<0.001) lesion volumes at 180 minutes, respectively.

Quantitative CBF Values Within ROIs

ROIs characterized as types 1, 4, and 5 were identifiable in all animals. ROIs 2 and 3 were not present in sMCAO animals and in only 4 and 6 eMCAO animals, respectively. The latter 2 tissue compartments were typically located in the outer rim...
of the critically hypoperfused tissue (data not shown). Figure 3 shows CBF values within investigated ROIs of both models. CBF did not significantly differ between contralateral ROIs over the course of the experiment \((P>0.05); \text{data not shown}\) and were thus averaged between regions and over time into a single value (Contra). Within the ipsilateral hemisphere of both models, CBF values significantly differed between but not within ROIs over time and were therefore averaged only within individual regions. Multiple comparison procedures showed in both models: (1) significantly lower CBF within all ipsilateral ROIs relative to Contra \((P<0.05)\), as well as (2) significantly different CBF between ipsilateral regions \((P<0.05)\). However, an intermodel comparison showed similar CBF values in the corresponding ROIs \((P>0.05)\).

### Quantitative ADC Values Within ROIs

Figure 4A and 4B show ADC values within the investigated ROIs of both models. ADC did not significantly differ between contralateral ROIs over the course of the experiment \((P>0.05); \text{data not shown}\) and were thus averaged over time into a single value (Contra). Relative to Contra, ADC in both models was: (1) not significantly different in ROI1 \((P>0.05)\), (2) significantly reduced in ROI4 (mismatch) from 60 minutes on \((P<0.05)\), and (3) significantly reduced in ROI5 (core) from 30 minutes on \((P<0.05)\). In eMCAO rats, ADC renormalized: (1) permanently (ie, up to 180 minutes after MCAO) by 90 minutes in ROI2, or (2) transiently at 60 minutes in ROI3. ROIs 2 and 3 could not be defined in sMCAO animals. Intermodel comparison showed significantly lower ADC values in the ischemic core of sMCAO animals at 30, 60, and 90 minutes relative to eMCAO rats \((P<0.05)\).

### Correlation of CBF With ADC Values Within ROIs

There was a significant correlation between ADC and CBF values in both models \((P<0.001)\), and there was a striking similarity between the correlation coefficient and the slope of the linear fit of both models (Figure 5).

### Discussion

Embolic models of MCAO have been used with increasing frequency to study neuroprotective drugs.\(^8,9,12\) Salvageable mismatch tissue cannot typically be identified beyond 3 hours in suture models of MCAO;\(^5\) however, prolonged treatment windows in eMCAO models suggest the presence of a penumbra for longer periods of time.\(^6,9,12\) However, a detailed investigation of acute differences in the spatiotemporal evolution of the ischemic lesion between both models is lacking.

The most important findings of this study are that the mismatch volume is larger and persists longer in eMCAO rats relative to sMCAO rats. Second, the same viability threshold can be used to define CBF lesions in both models. Third, the relationship between changes in CBF and ADC are very similar after eMCAO and sMCAO.

In the present study, eMCAO resulted in consistently larger abnormal perfusion volumes compared with permanent sMCAO. Because the extent of final infarct size highly correlated with abnormal perfusion volumes at all time points after sMCAO and eMCAO, the CBF viability threshold previously derived for the former model\(^13\) is also valid for the latter, excluding the possibility that the eMCAO perfusion lesion volumes were inaccurately derived. Parameters known to influence CBF, such as type and degree of anesthesia, arterial blood pressure, blood gases, and body temperature, are unlikely to have contributed to the difference in CBF lesion size as they did not differ between models. Additionally, there was no intermodel variation in basal CBF within...
the nonischemic left hemisphere, which makes it unlikely that this parameter was responsible for the aforementioned findings. Possible explanations for the intermodel variation in CBF lesion volume and spatial location include clot fragmentation with distal embolization or more complete proximal vascular occlusion. MR angiography performed in a subset of eMCAO rats showed occlusion of the PCA, which supports the former explanation.

Interestingly, though the final infarct volumes differed significantly between both models at 24 hours, the spatiotemporal evolution of the ADC-derived lesion volume did not differ between models within the first 3 hours after MCAO. In the acute phase, the abnormal diffusion volume increased within the first 90 minutes and essentially stopped growing at 2 hours after MCAO in both models. This cannot be simply explained by the constant intermodel differences in CBF lesion volume or a disparity in the correlation of ADC with CBF values between experimental groups. We analyzed the time course of CBF and ADC in 5 tissue compartments of the brain that are typically involved with focal cerebral ischemia. All 5 predefined tissue compartments were present in the eMCAO model and only 3 in the sMCAO model, demonstrating a more heterogeneous perfusion pattern in eMCAO rats compared with sMCAO rats, potentially because of the same variables responsible for the intermodel differences in the extent of CBF lesion volume. Tissue compartments with sustained and transient reversal of the diffusion/perfusion mismatch were typically located in the periphery of the critically hypoperfused tissue in eMCAO animals, and the ADC decrease in the ischemic core was more pronounced in sMCAO group compared with eMCAO group. Thus, we retrospectively hypothesize that the perfusion gradient from core to nonischemic tissue is less steep in the former group, which suggests a substantially slower growth of the threshold-derived ADC lesion beyond 3 hours, culminating in a much larger final infarct volume in eMCAO rats. However, this hypothesis could not be tested as imaging was not performed between 3 and 24 hours. In this respect, it is of note that the correlation between CBF and ADC was almost identical in both models, indicating that the viability threshold used herein for calculation of ADC lesions is valid for both experimental models.

This study demonstrated substantial intermodel differences in the temporal evolution of the diffusion/perfusion mismatch. The mismatch region in eMCAO rats was markedly larger up to 3 hours after MCAO compared with that in sMCAO rats. The diffusion/perfusion mismatch provides a volumetric estimate of the putative ischemic penumbra and the duration of its temporal existence and is thought to represent potentially salvageable ischemic tissue and is thus a major target for acute stroke therapies. Intermodel differences in the temporal evolution of the diffusion/perfusion mismatch may result in different therapeutic time windows for the same drug. It would be interesting to follow the mismatch evolution in the eMCAO model beyond the here-described 3 hours. Furthermore, delayed reperfusion or neuroprotective treatments in conjunction with observation periods beyond 24 hours could give insight into whether the peripheral parts of the critically hypoperfused areas after eMCAO are truly salvageable.

Acknowledgments
We like to thank Timothy Q. Duong and Qiang Shen for their support and helpful discussions.

References
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*Stroke*. 2006;37:1283-1287; originally published online March 23, 2006;
doi: 10.1161/01.STR.0000217223.72193.98

*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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