Image-Guided Method in the Rat for Inducing Cortical or Striatal Infarction and for Controlling Cerebral Blood Flow Under MRI

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Background and Purpose—Experimental models are essential for research on ischemic stroke, the second most common cause of death worldwide. The failure of clinical trials on neuroprotective treatment may be due in part to poor animal models. To push the translation of new therapies, we describe a new rat model that captures key elements of human brain ischemia. The model includes imaging and neurointerventional tools that represent the near future of clinical diagnosis and treatment of stroke.

Methods—Using Sprague-Dawley rats (n=26), we navigated a microwire with fluoroscopy and MRI guidance from the ventral tail artery to 2 different positions in the middle cerebral artery to establish local occlusion. Animals were scanned with 9.4-T MRI before occlusion, during ischemia, and after reperfusion.

Results—We detected stroke lesions, corresponding to the level of occlusion, in all animals by diffusion-weighted and T2 images. We measured lesion volume (mm3±SD) on T2 scans at 24 hours to be 23.2±29.8 in the somatosensory cortex group and 107.9±80 in the striatum group.

Conclusion—We present a new rat model for focal stroke with the possibility to cause lesions in different regions of the brain under fluoroscopic and MRI control. The model will be highly useful for extended studies on the ischemic penumbra, alterations in neural connectivity, and for investigating neurotransmitter-mediated events and biochemical changes in the hyperacute phase of brain ischemia. Also, the model uses clinical routine microcatheters facilitating superselective administration of therapeutics directly to the cerebral circulation. (Stroke. 2012;43:00-00.)

Key Words: acute stroke ■ animal models ■ brain imaging ■ focal ischemia ■ interventional neuroradiology

Stroke is the second most common cause of death and a major contributor to disability worldwide.1 Because of the aging population, the burden is estimated to increase considerably during the next 20 years.1 Because ischemic stroke in patients most often results from a thrombotic or embolic occlusion of the middle cerebral artery (MCA), rodent models have been developed to mimic human focal cerebral ischemia.2 The most widely used model, for focal cerebral ischemia in rats, is the intra-arterial suture occlusion of the middle cerebral artery (MCAO) by Koizumi et al modified by Longa et al.3,4 In the MCAO, a monofilament is inserted into the transected external carotid artery and advanced through the internal carotid artery until it reaches the anterior cerebral artery with the side of the monofilament occluding the MCA origin. The main limitation to this model is that blood flow to the anterior cerebral artery and posterior communicating artery is obstructed, resulting in large and variable infarctions of cortical and subcortical areas.5 Infarcts by the MCAO leave the animals in a highly variable and severe neurological condition hampering molecular studies on tissue recovery and functional testing.6–12 Furthermore, the MCAO model damages the hypothalamus resulting in hyperthermia with possible effects on pathological outcome.13

Another limitation to the MCAO is that the injury resembles the pathological state of a so-called malignant infarction with very poor prognosis instead of smaller human stroke that is possibly treatable to a higher degree.14 Ligation of the external carotid artery results in ischemic necrosis of mastication and hypopharyngeal muscles, which negatively affects outcome in behavioral testing.6 A plethora of alternative endovascular occlusion methods have been described.7,8,15–19 However, none of these permit controlled reperfusion of the
brain, a fundamental process for the pathophysiology in the vast majority of human strokes. Furthermore, reperfusion in ischemic stroke has become even more significant as thrombolytic therapy and modern neurointerventional tools aim at removing the occluding thrombus/thrombi by medical and mechanical means.20,21 The emerging technologies used in interventional radiology provide intriguing possibilities for organ-selective intervention and administration of therapies, especially regarding hard to reach organs such as the brain.22 These techniques have recently been applied successfully to improve modeling of human stroke in large animals.23–26 Although the use of large animals makes such procedures considerably easier technically, it causes other problems with ethics, costs, and thereby scalability for preclinical studies. Our hypothesis is that the use of microcatheter navigation would address the main obstacles presented by rat models of focal cerebral ischemia as mentioned previously.

Materials and Methods

Animal Model and Protocol

Twenty-six Adult male Sprague-Dawley rats (350–400 g, Scanbur B&K, Sollentuna, Sweden) were permitted food and water ad libitum until surgery. Anesthesia was induced using 4% isoflurane mixed with 100% O2 and subsequently maintained at 2% isoflurane. Animals were kept normothermic by means of a rectal thermistor coupled with a heating pad. A midline incision (5 mm) was made proximally on the ventral side of the tail. Subsequent steps were performed using microsurgery. The fascia covering the ventral artery was cut, and the exposed artery was ligated distally. Next, a 6-0 silk ligature was tied loosely around the proximal part of the exposed artery, and a microvascular clip was placed over the ventral artery. The artery was cut and a 0.020-inch “Marathon” hydrophilic microcatheter (ev3, Irvine, CA) carrying a microwire was introduced and advanced to the aorta. Reflow of blood into the microcatheter during navigation was prevented by constant flow of normal saline at a rate of 250 to 500 μL/h from a pressurized infusion system consisting of an intravenous pressure bag (commonly used in critical care and interventional cardiology).

Subsequent steps were performed using a Philips Allura Xper XD20 interventional x-ray system (Philips Medical Systems). Two groups underwent different interventional procedures: Group 1, the microwire was advanced on a 0.007-inch Hybrid microwire (Balt Extrusion, Montmorency, France) to the proximal common carotid artery. Next, the microcatheter was advanced on a 0.007-inch Hybrid microwire (Balt Extrusion, Montmorency, France) to the proximal descending aorta. Group 2 underwent different interventional procedures: Group 2, the tip of the microwire was placed in the MCA between the optic tract and the inferior cerebral vein (n = 8). Operation times were documented of the microwire was placed in the MCA between the optic tract and the inferior cerebral vein (n = 8). Operation times were documented. In Group 2, the microcatheter was advanced on a 0.007-inch Hybrid microwire to the proximal descending aorta. Next, the tip in the distal part of the MCA, MRI data were sampled every 10 minutes during 90 minutes of occlusion. After MRI analysis, the animals were returned to their cages with food and water ad libitum.

Monitoring

Physiological parameters, including oxygen saturation, CO2, and rectal temperature, were recorded every 10 minutes from the induction of anesthesia. Neurological status on a 0 to 3 grading scale according to Bederson et al28 was evaluated at 2, 4, and 24 hours postoperatively.

Magnetic Resonance Imaging

The in vivo MRI experiments were conducted using a horizontal 9.4-T magnet (Varian, Yarnton, UK) equipped with a 12-cm inner diameter gradient system with maximum gradient strength of 600 mT/m. A birdcage resonator was used for excitation (Rapid Biomedical GmbH, Würzburg-Rimpar, Germany) and a “rat brain” 4-channel phased array surface coil (Rapid Biomedical GmbH) served as the receiving coil.

Three-dimensional volumetric data of the entire brain were obtained using a fast spin-echo 3-dimensional pulse sequence resulting in mainly T2-weighted images.

Apparent diffusion coefficient maps were calculated from diffusion-weighted multislice spin-echo echoplanar imaging data with TR/TE 10 seconds/50 ms. Eighteen contiguous coronal slices of 1 mm thickness were acquired with a field of view of 38×19 mm² and a matrix size of 128×64. The diffusion encoding and decoding gradient pulses were applied along the left–right direction to yield a target b-value of 1100 s/mm².3

Data Analysis

Volumetric measurements were performed on 3-dimensional MRI data using OsiriX imaging software (OsiriX Foundation, Geneva, Switzerland). Volumes were built by manual tracing of the infarct perimeter. Manual tracing was performed in section intervals of 1 mm; the final volume was established through software interpolation and volume calculation. Two independent observers performed all measurements and volumetric data are expressed as mean values.

For signal intensity measurements in apparent diffusion coefficient maps, we used ImageJ software40 to select a rectangular area covering the lesion and a corresponding area in the ipsilateral cortex as shown in Figure 3D.

Anatomic correlation was performed by mapping the infarcted area displayed in 3-dimensional MRI data to stereotactic coordinate diagrams.27

In all animals, the anatomy of the MCA was determined by multiplanar reconstructions built by manual tracing from 3-dimensional MRI data using OsiriX imaging software (OsiriX Foundation). Classification of MCA anatomy was made according to Fox et al.18

Statistics

Comparisons between groups were performed by the Mann–Whitney U test. A probability value <0.05 was considered significant.

Results

In this article, we introduce a novel technique for microcatheter navigation from the ventral tail artery to the internal carotid artery, thereafter producing a focal cerebral ischemia by fluoroscopy-guided microwire occlusion at 2 different
positions in the MCA of the rat. Furthermore, we demonstrate the possibility to open and close a part of the MCA at the time the rat is located inside a MRI scanner and thereby the visualization of this procedure and the development of focal cortical ischemia by an ultrahigh field (9.4-T) MRI system.

We started with preliminary studies, in which we used fluoroscopy-guided microcatheter navigation and angiography to explore the vascular architecture from the ventral tail artery to the MCA (online-only Data Supplement Video I). Using the smallest available clinical routine microwires (0.007-inch to 0.010-inch) and microcatheters (0.016-inch to 0.020-inch), we entered the ventral tail artery and reached distal parts of the MCA with the microwire. After angiographic landmarking of the arterial route from the ventral tail artery to the MCA, this navigation was performed by fluoroscopy guidance only.

After concluding preliminary studies, showing the feasibility to apply endovascular intervention in the rat central nervous system with microwires, the second part of the study started. We used 2 groups that underwent different interventional procedures to perform a characterization study of the model. In Group 1, we navigated a 0.007-inch microwire to a position distal to the bifurcation of the MCA (n = 18; Figure 1; online-only Data Supplement Figure IA–B). In Group 2, we placed a 0.010-inch microwire in the main trunk of the MCA, between the optic tract and the inferior cerebral vein (n = 8; SI; online-only Data Supplement Figure IC–D). This position was maintained for 90 minutes in all animals, resulting in cortical infarctions with small variability according to MRI volumetric analysis.

Four animals in Group 1 (n = 18) were excluded due to subarachnoid hemorrhage secondary to perforation of the MCA. Two animals from Group 2 (n = 8) were excluded due to extensive infarction affecting the hypothalamic regions and hippocampus. Two animals, one from each group, were excluded due to absence of infarct at MRI analysis and 2,3,5-triphenyltetrazolium chloride staining. Thus, the relevant infarct incidence was 72% in Group 1 (n = 13) and 62.5% in Group 2 (n = 5). At 24 hours after reperfusion, we performed MRI analysis with T2-weighted fast spin-echo 3-dimensional pulse sequences and diffusion-weighted imaging (DWI). These imaging studies showed well-demarcated infarcts (Figure 2A–D). To determine the anatomic localization of the infarcts, we mapped sections from T2-weighted images to stereotaxic coordinate diagrams.27

In Group 1 (n = 13), all animals displayed cortical infarcts within the somatosensory cortex and 4 of 13 showed infarcts in the striatum (online-only Data Supplement Videos II and III). We observed infarcts predominantly in the S1 and S1bF parts of the somatosensory cortex with some involvement of the S2 area (Figure 2A, C). DWIs did not reveal any evidence of ischemia in the posterior communicating artery (PcomA) territory.

In Group 2 (n = 5), all animals had striatal infarcts and 2 of 5 also showed cortical infarcts. In the 2 animals with cortical involvement, the infarct was distributed to the cortical hemisphere involving somatosensory as well as motor areas.

The mean ± SD infarct volumes (mm³) for Groups 1 and 2 were 23.2 ± 29.8 and 107.9 ± 80, respectively (Figure 3). No
Thus, we demonstrate a minimally invasive technique for producing either isolated somatosensory cortical infarctions or combined striatal–cortical infarctions with reperfusion in the rat.

**Discussion**

This study shows that microwire navigation from the ventral tail artery to occlusive positions in different parts of the MCA is technically feasible and results in reproducible focal cerebral ischemia. Furthermore, the technique is truly minimally invasive and compatible with ultrahigh-field MRI systems to induce and monitor cerebral ischemia in-bore. For this study we used highly advanced equipment for fluoroscopy and angiography. However, along the project we discovered that angiography was unnecessary for navigation to the MCA. We realized that this method only requires basic fluoroscopy, a technique that is available worldwide at a low cost. With knowledge of vascular anatomy and its relation to skeletal landmarks, navigation from the medial tail artery to the occlusive position in the MCA is easily done by fluoroscopic guidance alone. Furthermore, the short surgical time allows high-throughput studies.

The intraluminal suture model (MCAO), originally introduced by Koizumi et al, is the most widely used rat model for focal cerebral ischemia. Despite numerous efforts to refine this model over the past decade, important features of human stroke remain to be successfully modeled. Human strokes are commonly much smaller than the infarcts produced in rat models. In a survey by Carmichael, human strokes were found to encompass 4.5% to 14% of the ipsilateral hemisphere compared with 21% to 45% in most rodent models. The large and highly variable subcortical infarction after MCAO is caused by obstruction of the blood
flow to the PcomA. In rats, the PcomA is the main tributary to the thalamus, hypothalamus, hippocampus, and substantia nigra. Because these important areas are infarcted, the animal suffers also from a variable level of increased body temperature, disturbed water homeostasis, and severe paresis, which all together infer bias in experimental studies and also are strong predictors of a poor prognosis of survival in humans with stroke. Truman et al. recently demonstrated the drastic differences in morbidity caused by different variants of the MCAO and concluded that the functional recovery after MCAO is attributed partially to effects of noncentral injury and that these confounding effects may mask effective treatment strategies. Also, the authors show that the animals lose up to 10% of the presurgical weight 3 to 4 days after MCAO and that this weight loss was not regained at 98 days postinjury. However, the weight gain curve followed the gain curve of normal rats from Day 3. In an additional experiment with our model (n = 4) we saw a 2% mean decrease in weight at 24 hours and a regain of presurgical weight at 48 hours postsurgery. This corroborates the findings in the Truman article that MCAO-associated weight loss is partly attributed to peripheral side effects.

Previously described models of focal infarction require craniotomy and most demand irreversible occlusion of the MCA. The MCAO has been referred to as “minimally invasive” although it requires extensive exploration of neck anatomy, transection of the external carotid, and ligation of several smaller branches of the internal carotid artery. In the endovascular method here presented, we entered the ventral tail artery by microsurgery to use it as an introducer. This procedure is easy to perform leaving the animal with minimal discomfort and it also allows repeated procedures in the same animal. The distal part of the tail does not suffer from ischemia because the 2 lateral tail arteries take over the supply through numerous anastomoses. Thus, we present a truly minimally invasive model that produces small focal cerebral infarcts limited to the somatosensory cortex by selective occlusion of the main posterior branch of the MCA. This was, in part, possible due to the low variability of the gross anatomy of the MCA, as previously demonstrated by Fox et al. It was possible to navigate a 0.007-inch neurointerventional microwire to the main posterior branch of the MCA without causing infarcts in the PComA territory or in
other parts of the basal ganglia. Cortical areas supplied by distal parts of the posterior MCA branch were also unaffected indicating significant leptomeningeal collateral supply from the posterior and anterior cerebral arteries. Although we produced consistent infarction in the cortical area corresponding to the tip of the microwire, we noted occasional minor infarction of the putamen in 2 of 13 animals in Group 1 caused by occlusion of a few lenticulostriate branches from the proximal segment of the MCA.

We were also able to produce striatal infarction by selective occlusion of multiple lenticulostriate branches arising from the M1 segment of the MCA. With a 0.010-inch microwire lodged in the proximal MCA, before the rhinal fissure, we saw consistent ischemia in the ipsilateral lenticulostriate territory with occasional cortical involvement, probably depending on interindividual variations in leptomeningeal collateral pattern. In this group, however, 2 of 8 rats developed infarcts in parts of the PcomA territory, indicating obstruction of blood flow due to the larger diameter of this microwire.

In human stroke, the type and extent of behavioral deficit are dependent on the injury caused by specific functional circuitry such as different parts of the somatosensory cortex.36 Recovery after an ischemic event involves reorganization and reactivation of adjacent cortical sites. To study rehabilitative therapies, adequate modeling of neural repair in human stroke requires that animal stroke models produce injury in defined brain circuits. Thereby it will be possible to study molecular and cellular events involved in recovery of the area adjacent to the infarct. The MCAO results in cortical infarcts involving large parts of motor and somatosensory cortex as well as subcortical circuits. By navigating microwires of different sizes to different vascular positions, we were able to produce small infarcts confined to the somatosensory cortex as well as strictly striatal infarcts with a small overlap. This was reflected in the neurological testing and postoperative weight assessment. The majority of the animals were in excellent condition displaying normal behavior and did not lose weight during the experiment. This stands in sharp contrast to the animal condition in the MCAO model in which gross motor–sensory functions, temperature, water, and weight homeostasis are severely disturbed. The presented model will allow more specific functional and molecular analysis of tissue regeneration and behavior. Moreover, experimental treatment outcomes will be more readily assessed with a more defined injury.

Another major limitation of current experimental stroke models is the difficulty to combine them with in-bore occlusion of cerebral vessels. In-bore MCAO has been described,37–39 although methods for this are technically very challenging, thus reducing reproducibility. The technique in the current study proved highly advantageous for performing in-bore occlusion. After positioning the microwire in the proximal segment of the MCA we were able to place the animal inside an ultrahigh field imaging magnet with relative ease. Wire manipulation followed by imaging made it possible to exactly navigate and thereafter, with MRI, confirm the position of the occluding microwire. Earlier studies have shown a significant apparent diffusion coefficient drop as early as 3 minutes after ischemia onset.37 Because we did not see any changes by DWI during shimming, radiofrequency calibration, and baseline imaging, a process that needed approximately 30 minutes, we conclude that the blood flow was not significantly impaired until moving the microwire to its final position.

In-bore experiments with ultrahigh field magnet and metal microwires are technically contradictory. However, of the 2 most frequently used alloys in microwires, stainless steel and nitinol (nickel–titanium), nitinol is nonmagnetic. Thus, navigation with a nitinol microwire was possible without any problems, the animals tolerated the experiment well, and, at 24 hours, MRI analysis showed T2 signal loss throughout the MCA lumen proving preserved blood flow. Importantly, the microwire did produce discrete artifacts that allowed exact determination of its position without limiting infarct analysis.

In conclusion, the method presented here has several advantages. It is minimally invasive and fluoroscopic control allows selective endovascular occlusion of the MCA, resulting in small focal infarcts confined to the somatosensory cortex or the striatum, depending on choice. The technical equipment required is available worldwide at a low cost and high-throughput experiments are feasible because surgical times are short. Compared with other rat models, these infarcts more resemble common human stroke and the technique also permits avoidance of infarction of the important PcomA territory, which in rats includes the thalamus, hypothalamus, hippocampus, and substantia nigra. This is highly important because the confounding effects by extracranial tissue damage in the MCAO have been found to be drastic. From an ethical standpoint this model significantly limits the suffering caused by side effects secondary to large unspecific infarct lesions, surgical exploration of the neck, and ligation of the external carotid artery. Because the lesion is significantly smaller than the lesion produced by the MCAO, more structures in the lesioned hemisphere are conserved, thereby providing extended possibilities to study the infarct penumbra and the effects of hypoperfusion.

Also, the method is easily compatible with ultrahigh field magnet imaging equipment, presenting MRI-controlled microwire navigation to control occlusion and reperfusion. The microcatheter that carries the microwire can also serve as a working channel wherein therapeutic agents may be administered in a targeted fashion.

Appendix

Scientific Committee of Fighting Stroke (Uppdrag Besegra Stroke)

Nils Wahlgren (Chair), Niaz Ahmed, Maaret Castrén, Ulf Eriksson, Jonas Frisén, Ulf Hedén, Staffan Holmin, Åke Sjöholm, Mikael Svensson, and Mia von Euler.

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SUPPLEMENTARY FIG 1
SUPPLEMENTARY FIG 2
FIGURE LEGENDS

Supplementary Fig.1
Frontal (a, c) and side (b, d) view X-ray images of microwire placement in the middle cerebral artery (MCA). a,b) Group 1 – placement of a 0.007” platinum-coated nitinol microwire in the posterior branch of the MCA. c,d) Group 2 – placement of a 0.010” platinum-coated stainless-steel microwire placed in the proximal segment of the MCA.

Supplementary Fig.2
Curved multiplanar reconstruction, from MRI T2 images, of the middle cerebral artery anatomy (MCA). The MCA is visualized from the branching from the circle of Willis to the parietal cortex. An ischemic lesion corresponding to the posterior branch of the MCA is seen as a hyperintense signal.

VIDEO LEGENDS

Supplementary Video 1
Digital subtraction angiography images of the cerebrovascular anatomy in a rat (frontal view). The contrast media is injected into the internal carotid artery through a 0.020” microcatheter.

Supplementary Video 2
3D volumetric MRI data from T2 images, obtained by a fast spin-echo 3D pulse sequence, displaying a small cortical infarct in an animal from Group 1.

Supplementary Video 3
3D volumetric MRI data from T2 images, obtained by a fast spin-echo 3D pulse sequence, displaying striatal infarction in an animal from Group 2.