Effect of Suture Properties on Stability of Middle Cerebral Artery Occlusion Evaluated by Synchrotron Radiation Angiography

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Background and Purpose—The intraluminal suture technique for producing middle cerebral artery occlusion in rodents is the most commonly used method for modeling focal cerebral ischemia associated with clinical ischemic stroke. Synchrotron radiation angiography may provide a novel solution to directly monitor the success of middle cerebral artery occlusion.

Methods—Twenty adult Sprague-Dawley rats for middle cerebral artery occlusion models were prepared randomly with different suture head silicone coating. In vivo imaging was performed at beam line BL13W1, Shanghai Synchrotron Radiation Facility, Shanghai, China.

Results—Silicone-coated suture was superior to uncoated suture for producing consistent brain infarction. Additionally, silicone coating length was an important variable controlling the extent of the ischemic lesion: infarcts affected predominantly the caudate–putamen with large variability (<2 mm), both the cortex and caudate–putamen (2–3.3 mm), and most of the hemisphere, including the hypothalamus (>3.3 mm).

Conclusions—Synchrotron radiation angiography provides a useful tool to observe hemodynamic changes after middle cerebral artery occlusion, and the physical properties of suture are critical to the success of the middle cerebral artery occlusion model. (Stroke. 2012;43:888-891.)

Key Words: angiography ■ middle cerebral artery occlusion ■ synchrotron radiation

The focal cerebral ischemia model involves the occlusion of the middle cerebral artery (MCAO) and typically results in localized brain infarction, which recapitulates many of the pathophysiological and histopathologic features of stroke. The most common technique for MCAO is the intraluminal filament model. However, 1 limitation of the suture model is its high variability in infarct size. Factors that contribute to such variation include differences in animal strain and weight, method of anesthesia, blood pressure, brain and body temperature, brain vascular anatomy, suture material, duration, and site of occlusion. Physical properties of the filament are important because it is the key factor that affects lesion volume; however, there is no direct evidence to confirm this principle.

Regional cerebral blood flow can be measured using autoradiography and laser Doppler flowmetry to confirm successful MCAO, but neither method can dynamically monitor changes of cerebral blood flow in deeper tissue during ischemia–reperfusion. Synchrotron radiation angiography (SRA) may represent a novel solution to directly and dynamically monitor MCAO. In the present study, we used SRA to examine if the coating length was critical for producing a highly reproducible stroke model.

Materials and Methods

Experimental Groups

Animal procedures were approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University, Shanghai, China. Twenty male Sprague-Dawley rats (SLAC, Shanghai, China) weighing 270 to 350 g were divided into 4 groups randomly (n=5/group). The sutures with different physical properties were used to produce the MCAO model: Group 1, 4-0 monofilament nylon suture without silicone coating; Group 2, 4-0 suture with a 2.0-mm silicone coating length; Group 3, 4-0 suture with a 2.0- to 3.3-mm silicone coating length; and Group 4, 4-0 suture with >3.3-mm silicone coating.

Surgical Procedure

Animals were anesthetized using 50/10 mg/kg ketamine/xylazine (Shanghai Pharmaceuticals Holding Co, Shanghai, China) intraperi-
The middle cerebral artery was occluded as described previously. A customized “T”-shaped PE-10 catheter was carefully inserted into the internal carotid artery and fixed throughout the subsequent imaging procedure.

**Synchrotron Radiation Imaging**

The experiment was conducted at beam line BL13W, Shanghai Synchrotron Radiation Facility. The beam current was 145 mA and x-ray energy was 33.7 keV. A charged coupled device camera (13 μm/pixel) was used to obtain real-time angiographic imaging. Images were snapped at 30 frames/second. Animals were placed in the right or left lateral decubitus position on a flexible platform. A total of 175 mg/mL nonionic iodine contrast agent (Omnipaque; GE Healthcare) was administered through a PE-10 catheter at an injection rate of 100 μL/s. The total injection volume was <1 mL per rat.

**Infarct Volume Analysis**

Rats were euthanized 24 hours after MCAO. Brains were isolated and cut into 2-mm-thick coronal slices and stained with 2% wt/vol 2,3,5-triphenyltetrazolium chloride (Sigma, St Louis, MO). Infarct volume was measured using National Institutes of Health Image J software (Bethesda, MD) and calculated as described.

**Statistics**

Data were presented as means±SD. Statistical differences among groups were determined by 1-way analysis of variance with repeated measures. A value of P<0.05 was considered to be statistically significant.

**Results**

**SRA of the Rat Brain**

The entire vasculature of the left hemisphere including pterygopalatine artery, internal carotid artery, middle cerebral artery (MCA), anterior choroidal artery, posterior cerebral artery, and small (13 μm diameter) arteries and small veins was detected. The distance between the proximal posterior cerebral artery/MCA, anterior choroidal artery/MCA, and MCA/anterior cerebral artery is 2.23±0.25 mm, 1.50±0.18 mm, and 1.58±0.35 mm, respectively (n=14). The distance between branches of the MCA in the bifurcated MCA is 1.47±0.05 mm (n=6).

Figure 1. Synchrotron radiation angiography images were taken immediately after the injection of contrast agent. Images shown (top to bottom) were taken at 50 ms, 125 ms, 200 ms, and 275 ms. Normal cerebral vasculature (left); unsuccessful middle cerebral artery occlusion (MCAO) using 4-0 suture without coating (center) and successful MCAO using silicone-coated 4-0 suture (right) are detected.

Figure 2. Synchrotron radiation angiography images showing the extent of middle cerebral artery (MCA) occlusion using silicone-coated sutures with different coating lengths. The suture can be visualized in these images as a thin white line in the internal carotid artery. The MCA was only partially occluded when the suture head was not coated (A) or the coating length was <2 mm (B). The MCA was successfully occluded when the coating length was 2.0 to 3.3 mm without affecting the hypothalamic artery (HTA), posterior cerebral artery (PCA), and anterior choroidal artery (AChA; C). The MCA was occluded when the coating length >3.3 mm together with AChA/PCA, and HTA (D).
Effect of Suture Properties on MCAO
We first tested the effect of 4-0 nylon suture with or without silicone coating. We found that contrast enhancement was still detected in the MCA when an uncoated suture was used (Figure 1). Next, we examined whether the length of silicone coating would influence its ability to block the backflow from communicating arteries. We demonstrated that the MCA cannot be occluded when the suture tip was not coated; when the silicone coating length was <2 mm, the MCA was partially blocked; when the silicone coating length was 2.0 to 3.3 mm, the MCA was completely occluded; when the coating length was >3.3 mm, arteries including the anterior choroidal artery/posterior cerebral artery and hypothalamic artery were occluded (Figure 2). Further study revealed the correlation between suture coating length and infarct size (Figure 3). When the coating length was 2 to 3.3 mm, ischemia affected both the cortex and caudate–putamen with very small deviation. Additionally, this coating length was also optimal for survival (2–3.3 mm [100%] versus >3.3 mm [25%]; P<0.05).

Discussion
The ability to monitor vessel occlusion in real time may ensure uniformity in the location and completion of occlusion. Recently developed techniques such as MR angiography provide in vivo methods for imaging the cerebral circulation in rodents. However, the resolution of MR angiography is relatively low, which is likely to preclude its use for monitoring of MCAO in rodents. SRA provides a useful tool to study changes in cerebral blood flow and vascular morphology in real time in the rodent brain.

Early intraluminal MCAO models used silicone–rubber-coated and flame-blunted monofilaments. However, variability in infarct size led to modification of sutures with different coating materials. Additionally, heat-blunted monofilaments were found to be associated with a variable infarction volume. Methods for comparing the effects of different occlusion techniques have been lacking. Our results show that in a certain range of weight (we used 270- to 350-g rats), the length of suture coating is a critical factor in generating reproducible infarct after MCAO. However, several disadvantages need to be considered. The imaging window of SRA is small (4×40 mm). Additionally, the SRA device is not universally available. Nevertheless, SRA provides a valuable technological adjunct to the study of experimental stroke.

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Disclosures
None.

References


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