Middle Cerebral Artery Occlusion Model of Stroke in Rodents: a Step-by-Step Approach

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Abstract

Stroke is one of the leading causes of morbidity and mortality in developed countries and an immense amount of medical care resources are devoted to combat the poststroke debilitating consequences. The key to develop effective and clinically applicable treatment methodologies is a better understanding of the pathophysiology of the disease, including the root causes and targets for pharmacology. Developing these foundations requires the use of standard animal models that mimic the physicochemical process of the diseases that can reliably replicate results in order to test and fine-tune therapeutic modalities. Middle cerebral artery occlusion (MCAO), endothelin-1-induced ischemic stroke, photothermolysis, devascularization, embolization, and spontaneous infarction using hemorrhage are some examples of different animal models. Reliability of MCAO has been proved and due to the ability to induce reperfusion similar to tissue plasminogen activator (tPA) therapy, this model is widely used in preclinical studies.

Here, we describe a detailed methodology on how to develop MCAO stroke in rodents using intra-arterial insertion of filament to occlude the middle cerebral artery. This approach allows for the study of a wide array of basic pathophysiology mechanisms, regenerative medicine and rehabilitation therapy.

Keywords

Animal model; middle cerebral artery; occlusion; stroke

Introduction

Cardiovascular disease and stroke are the leading causes of disability in western society and is estimated to have a total direct and indirect cost of about $315.4 billion in 2010 [1,2]. In the United States, stroke has had a mortality rate of 15%, and among the survivors aged 65 years and older, 26% are institutionalized at 6 months after stroke, 50% suffer from hemiparesis and 30% require assistance to walk [3,4]. Middle cerebral artery (MCA) stroke, affecting approximately one person every 40 seconds, often results in significant and permanent disability [5].

Improving care for stroke relies critically on the use of animal research models. As suggested by the stroke treatment academic industry roundtable (STAIR), agents for prehospital testing should show evidence of safety in
stroke models, among other important steps [6]. Several stroke animal models, including rats, mice, pigs, dogs, cats, and nonhuman primates, have been investigated. As highlighted by stem cell therapies as an emerging paradigm in stroke (STEPS), rat models of stroke are considered the species of choice for testing restorative therapies [7]. This superiority might be due to (1) similar neurovascular branching to humans, (2) having reproducible results, (3) relatively low cost to nurture, and (4) practical size for monitoring and studying neuropathology [8]. Interstrain differences are critical in producing consistent ischemic damage and should be considered when comparing treatment outcomes in rat models of strokes. Thereby, maintaining consistency in choosing one strain (i.e., Sprague–Dawley, Wistar, Long Evans) enables the scientist to interpret the result of different studies.

The MCA is commonly affected in clinical ischemic stroke, and hence, occluding this major vessel downstream of the internal carotid artery in rats replicates clinical cerebral ischemia. The MCA can be accessed via an approach through either the internal or the external carotid artery and be occluded temporarily or permanently. Infarcts induced by this approach often comprise both striatal and cortical damage. The intraluminal filament middle cerebral artery occlusion (MCAO) stroke model in rats was initially developed by Koizumi in 1986 and modified by Longa [9,10]. Temporary insertion of a filament in the MCA, which is later removed after the desired period of ischemia, produces a transient MCA territory ischemia followed by the restoration of blood circulation. Alternatively, leaving the filament in the MCA or clipping the artery results in permanent occlusion.

Advantages of this method include the ability to avoid craniotomy (and subsequently prevent its influence on blood–brain barrier permeability and intracranial pressure), the production of a focal occlusion, and high-throughput potential. Disadvantages include subarachnoid hemorrhage, tracheal edema, and paralysis of muscles of mastication and swallowing due to injury of the external carotid artery (ECA) [11].

Many factors can affect the reproducibility and anatomical accuracy of this technique and it is important to develop a standardized protocol and appropriate surgical techniques. This manuscript describes intraluminal filament MCAO stroke model in mice in order to improve rates of experimental success for future studies.

**Materials**

Materials included in nonsurgical supplies, surgical tools and supplies, and tools for infarct volume measurement are described as follows:

1. **Nonsurgical supplies**
   1. Anesthesia machine (VetEquip Inc.)
   2. Isoflurane (Baxter International Inc.)
   3. Laser Doppler flowmetry machine (PeriFlux System 5000)
   4. Flexible microtip (MT B500-0 Straight Microtip)
   5. Rectal temperatures monitor (World Precision Instruments).
   6. Thermal blanket (World Precision Instruments)
   7. Surgical/dissecting microscope (Leica Stereo Microscope MZ6)
   8. Fiber optic illuminator (Fiber Optic illuminator FO1–150)
   9. Filament for MCA occlusion: 7-0 surgical Nylon monofilament suture (Ethicon)
   10. Thermal cautery unit (World Precision Instruments)
   11. Liquid silicone rubber (Silicone RTV adhesive, World Precision Instruments).

2. **Surgical tools and supplies**
   1. SuperCut Iris scissors, straight (World Precision Instruments)
   2. Vannas microscissors, straight (World Precision Instruments)
   3. Dumont forceps (fine tip, 45°bent tips, World Precision Instruments)
   4. Bipolar coagulation unit (High Frequency Desiccator 900, World Precision Instruments)
   5. Microvessel clip (World Precision Instruments)
   6. Sterile cotton tips
   7. Wipes
   8. Gauze sponges
   9. Suture (Ethicon, 4-0 Silk and 7-0 Nylon sutures for ligation and occlusion, respectively)
10. Tissue adhesive (Vetbond, 3M Company)
11. Surgical scrub including Betadine, 70% ethanol, and sterile 0.9% saline.

3. Tools for infarct volume measurement
   1. 2% 2,3,5-triphenyltetrazolium chloride (TTC) solution (Sigma-Aldrich). (Note 1)
   2. Brain matrix slicer (Zivic-Miller Lab.)
   3. Digital camera
   4. Image J software (ImageJ 1.42q software, U.S. National Institutes of Health)

Methods

1. Surgical and suture preparation
   1. Sanitize surgical area and equipment with 70% ethanol and sterilize all surgical tools.
   2. Take desired nylon suture (7-0 nylon) and blunt 1–2 mm of suture tip with thermal cautery unit (length of 11–13 mm).
   3. Coat the blunted suture tip with liquid silicone rubber and let the silicon rubber dry at room temperature to achieve a tip diameter range of 0.2–0.3 mm.

2. Animal preparation
   1. Examine animals for gross abnormalities and behavioral deficits prior to anesthesia and surgery.
   2. Weigh each animal and then anesthetize in an appropriate-sized induction chamber by using 5 L/min isoflurane flow rate in 30% O₂/70% N₂O mixture. Decrease flow rate to 1 L/min for maintenance.
   3. Place the animal in a prone position on a gauze pad lying over a thermal blanket to maintain adequate body temperature of 37 ± 0.5°C, as monitored using a rectal temperature probe, while administering the anesthesia described in and during the surgical procedure.
   4. Shave head fur using an appropriate size hair clipper.
   5. Apply small amount of lubricant ophthalmic ointment to both eyes to prevent eye desiccation during surgery.
   6. Make a midline incision (around 1 cm) on the skin over the skull from between the nasion in front of the superior nuchal line in back. Dissect tissue until the surface of skull can be felt with forceps to allow for probe placement.
   7. Affix a flexible microtip (MT B500-0 Straight Microtip; 0.5 mm diameter) perpendicular to the (right or left) parietal bone of the skull; 1 mm posterior and 5 mm lateral to the bregma point (Fig. 1) in order to monitor blood flow in MCA territory using laser Doppler flowmetry (Note 2).
   8. After firm attachment of the microtip to the skull, place the animal in a supine position and support the neck with a soft pad while also providing enough space for the microtip.
9. Tape the forepaws and the hind probe paws to the operating surface and the rectal probe to the tail to secure its position.

3. Cervical dissection and MCA occlusion method

In the following sections, both the Koizumi and Longa methods for MCAO will be described [9,10]. First, the initial steps that are common for both procedures will be explained. Next, procedures specific to each method will be described.

1. Shave the fur on the anterior neck over the front part of the neck and apply 70% alcohol and Betadine to sterilize the incision area.

2. Using sharp scissors make a midline incision through the skin from the manubrium of sternum to the area below jaw (Note 3).

3. Under microscope guidance, gently divide the right and left submandibular glands and dissect the gland on the side of vascular surgery (right or left) to release it from the surrounding fascia. Then retract the gland cranially and clamp it to the sternocleidomastoid muscle of the corresponding side. The carotid triangle should be visible at this point (Fig. 2).

4. Divide the exposed part of the omohyoid muscle to uncover the underlying carotid sheath.

5. Dissect the carotid sheath, carefully isolate the common carotid artery and separate it from the vagus nerve, which is located lateral to it (Note 4).

6. Proceed to either MCA occlusion based on Koizumi’s method or Longa’s method as described in the following sections.

Koizumi’s MCAO method

In Koizumi’s MCAO method, the steps to be followed are given below:

1. Place three loose collar sutures (4-0 silk) around the common carotid artery (CCA) (Fig. 3).

2. Find the external carotid artery (ECA), isolate, and then cauterize it very close (2–3 mm distal) to CCA bifurcation (Note 5).

3. Firmly tie the most proximal collar suture on the CCA to permanently occlude the blood flow. Successful occlusion is verified by ≥60% laser Doppler flowmetry (LDF) (Note 2).

4. Place a vessel clip on CCA just before its bifurcation to prevent retrograde flow at the time of arteriotomy.

5. Cut the CCA between the proximally ligated suture and the vessel clip using a microscissors. The arteriotomy should be closer to the ligation to leave a longer stump of CCA for filament insertion.

6. Introduce the heat-blunted and silicone-coated 7-0 nylon suture into the CCA stump at the arteriotomy site and advance the suture till it reaches the vessel clip.
7. Before removing the vessel clip to further advancing the occluding suture towards the internal carotid artery (ICA) and to prevent back flow leakage through CCA, gently tighten the other two remaining loose collar sutures around the CCA between the arteriotomy site and the vessel clip.

8. Proceed to perform the rest of the procedure as described in common MCAO procedure.

**Longa’s MCAO method**

In this method, steps involved are described below (Fig. 4):
1. Find the ECA and dissect it as far close to its bifurcation as possible. This will avoid bleeding from ECA branches, such as the lingual and maxillary arteries.
2. Cauterize the ECA near its distal branches and divide it with microscissors.
3. Place two loose collar sutures (4-0 silk) around the proximal part of the ICA just above the CCA bifurcation.
4. Temporarily close the CCA with a vessel clip. Successful closure can be verified by ≥60% LDF reduction in baseline cortical perfusion values (Note 2).
5. Temporarily close the ICA with a vessel clip above the two loose collar sutures to prevent retrograde flow at the time of arterotomy.
6. Using microscissors, perform an arteriotomy in the reflected ECA close to the stump.
7. Introduce the heat-blunted silicone-coated 4-0 nylon suture into the ECA stump at the arteriotomy site and advance the suture until it reaches the vessel clip on ICA.
8. Before removal of the vessel clip around ICA to further advance the occluding suture and to prevent back flow leakage through ICA, gently tighten the other two remaining loose collar sutures around the proximal part of ICA, with special care not to damage the arterial wall.
9. Proceed to perform the rest of the procedure as described in common MCAO procedure.

**Common MCAO procedure**

The steps involved in common MCAO procedure is described as follows:

1. Gently advance the occluding suture through the ICA toward the cranial base until feeling a mild resistance. At this stage, a drop in cerebral blood flow value (~80% of the baseline blood flow value) detected by LDF measurements indicates MCA occlusion.
2. Depending on study aims, the occluding suture may be withdrawn gently after a certain time period to model clinical reperfusion following tPA treatment. Sufficient cerebral ischemia can be achieved after a 45-minute transient occlusion.
3. Seal the arteriotomy site after suture filament withdrawal to avoid post-surgical bleeding.

This involves tightening of the collar sutures around the CCA in Koizumi’s method and cautery of the base of ECA in Longa’s method followed by loosening and removal of the collar sutures around the proximal portion of the ICA to restore blood flow.

4. After confirming that none of the vessels are leaking, return the submandibular gland and the sternocleidomastoid muscle (SCM) to their normal position over the operative field and close the wound edges in the cervical and head area using sufficient amount of tissue adhesives. If long term survival of the animal is intended, it is highly recommended to use absorbable sutures or metal staples to close the wounds.

5. To compensate for any blood loss during surgery, inject 1 ml of warm saline subcutaneously. Saline could be supplemented with a dose of appropriate analgesics, such as buprenorphine (at 0.05–0.1 mg/kg) to lower animal pain and discomfort while recovering.

6. To prevent hypothermia, place the animal in a warm recovery area in a secure cage with free access to soft food and water.

### 4. Poststroke neurological assessment

To evaluate the level of neurological deficits due to stroke, use the following five-point scale when the animal attains full consciousness:

0. No observable deficits
1. Inability to extend contralateral forepaw (mild focal neurological deficit)
2. Circling toward contralateral to infarct (moderate focal neurological deficit)
3. Falling towards contralateral to infarct (severe focal neurological deficit)
4. Low level of consciousness with no spontaneous movement

### 5. Sample staining and brain infarct volume measurement

1. Prepare a 2% TTC solution (e.g., dissolve 2 g of TTC in 100 ml of phosphate-buffered saline).
2. Sacrifice the animal after deeply anesthetizing with isoflurane or other approved anesthetics.
3. Gently and carefully remove the brain and place in a mouse brain matrix slicer to obtain 2 mm coronal sections of the cerebrum.

4. Incubate the coronal brain sections in 2% TTC solution at 37°C for 20 minutes. After staining with TTC, infarcted tissue will remain unstained (white), while viable tissue will be strongly stained (brick red) (Fig. 5).

5. Place the stained serial slices in order and photograph them, using a digital camera.

6. By using Image J software, measure the area of infarct, the area of ipsilateral hemisphere, and the area of the contralateral hemisphere in each slice.

7. Sum up the values obtained for representative areas in all slices and multiply it by slice thickness to calculate the estimated stroke volume, ipsilateral volume, contralateral volume, and total volume.

8. To correct for edema, use the following equation (Note 6):

\[ \text{Infarct volume percentage} = \left[ \frac{\text{C.H. Volume} - (\text{I.H. Volume} - \text{Infarct Volume})}{\text{C.H. Volume}} \right] \times 100 \]

Where C. H. volume is contralateral hemisphere volume, and I. H. volume is ipsilateral hemisphere volume.

Notes

1. Prepare 2% TTC solute by dissolving TTC powder in phosphate-buffered saline. Suggestion: consider keeping small aliquots in 4°C fridge so that solution will not be exposed to light and can be used for each experiment.

2. LDF probe should be well fixed to prevent disturbance during surgery. This procedure allows for the measurement of relative cerebral blood flow (rCBF). Values measured prior to and following the surgery allows for calculation of relative changes in blood flow. To improve consistency and comparability and as suggested by STAIR, only mice with overall ≥60% flow reduction during the ischemic period (measured by transcranial LDF) are included in the experiment [12].

3. Consider the balance between a large versus a small incision. Larger incisions allow for better visualization of cervical structures but increases surgical burden on the experimental animals. With improved surgical proficiency, incision size can be minimized and may subsequently help to reduce surgical morbidity and mortality.

4. In comparison to other cervical vasculature, the CCA can be visualized as the pulsating vessel with a sturdy consistency upon manipulation, located within the carotid sheath and branching into the ICA and ECA distally.

5. ECA can subsequently be divided with microscissors. To avoid inadvertent peripheral damages at the cauteri-
zation site, the power should be set at minimum using the power knob for all cauterizations.

6. Edema is a key pathophysiological mechanism in ischemic stroke [13]. Unfortunately, swelling of infarcted tissue may cause overestimation of ipsilateral hemisphere and infarct size. As a result, the equation described uses the contralateral hemisphere of each individual animal brain as a control for its ipsilateral hemisphere.

References