



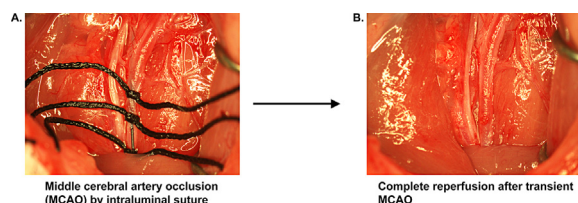
Transient middle cerebral artery occlusion with complete reperfusion in spontaneously hypertensive rats



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GRAPHICAL ABSTRACT



ABSTRACT

Middle cerebral artery occlusion (MCAO) by the intraluminal suture method is widely used to model ischemic stroke in rats. Current methods include transection or ligation of the external carotid or common carotid artery and thus result in partial restoration of perfusion after transient MCAO. Since incomplete reperfusion may influence recovery and thus confound studies of the impact of neuroprotective compounds and therapies on outcomes after stroke, we have devised a novel method to induce transient MCAO with complete reperfusion. Advantages of the method include:

- MCAO is achieved through insertion of an intraluminal suture into the internal carotid artery through the common carotid artery.
- At the end of the occlusion period, the suture is withdrawn and the incision in the common carotid artery is closed with cyanoacrylate tissue adhesive and complete reperfusion is established.
- No residual subcutaneous sutures remain during recovery.
- Vasculature is restored to the preoperative state.

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Method

Required materials

Spontaneously hypertensive rats (SHR) (male, 290–300 g, Harlan or Charles River Laboratories)
MCAO sutures (Docol Corporation, 403923PK10)
4–0 braided silk sutures (Ethicon, A183H)

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S&T vascular clamp, 7 mm length, 1 × 3.5 mm jaws (Fine Science Tools, 396-01)
Colibri Retractor, 1.5 cm spread, 9 × 3 × 6 cm teeth (Fine Science Tools, 17000-03)
GLUture topical tissue adhesive (32406-01)

Method details

All animal procedures were approved by the University of New Mexico Institutional Animal Care and Use Committee and were in compliance with federal guidelines. Animals were anesthetized with 2% isoflurane inhalant during all surgical procedures.

Step one: preparation of the common carotid artery (CCA) for insertion of the occluding suture

The animal is anesthetized with 2% isoflurane and immobilized in the supine position on a surgical table with a 15 ml tube placed under the neck to facilitate visualization of neck vasculature. The neck is shaved, disinfected with povidone-iodine solution, and cleaned with 70% ethyl alcohol. A shallow 25 mm incision is made along the midline from the base of the mandible to the sternum. The right CCA is carefully exposed while avoiding injury to soft tissues and nerves as described elsewhere [1]. The sternohyoid and sternomastoid muscles are retracted and the CCA is separated from the vagus nerve (Fig. 1). The CCA is ligated with a 4–0 silk suture with a single knot 6–7 mm caudal to the bifurcation of the CCA into the external carotid artery (ECA) and internal carotid artery (ICA). The knot should be sufficiently tight to occlude the CCA but not overly tightened so as to prevent injury to the CCA and facilitate later removal of the suture. A vascular clamp is placed on the CCA immediately caudal to the bifurcation. Two 4–0 silk sutures are tied loosely around the CCA between the first suture and the vascular clamp (Fig. 2).

Step two: middle cerebral artery occlusion (MCAO)

A punctate incision is made in the ventral wall of the CCA with a 25G needle 5 mm caudal to the clamp and a 4–0 nylon suture (Doccol Corporation) 30 mm in length with a 2–3 mm silicone-coated tip (0.39 mm diameter) is advanced into the CCA lumen to the clamp. The lower silk suture is secured around the occluding suture to prevent bleeding (Fig. 3). The clamp is removed and the suture advanced into the ICA until resistance is felt. In the correct position, the end of the suture should be roughly at the caudal end of the surgical field. If resistance is felt while much of the suture is outside the surgical field, the suture may have entered the pterygopalatine artery. If this occurs, the suture is pulled back to the bifurcation and reinserted while attempting to direct it toward the midline. To ensure proper placement of the occluding suture once resistance is felt, the suture is grasped with forceps, bent toward the midline, and gently pushed forward into the ICA until flexion of the suture can be visualized through the lateral wall of the ICA (Fig. 4). Care must be taken during this step to not insert the suture past the point of visualizing flexion through the ICA or the ICA may be punctured by the suture and result in subarachnoid hemorrhage. Additionally, flexion of the occluding suture should be visualized only once to prevent stretching of the ICA which can result in increased variability in infarction. After flexion of the suture has been observed, the suture is immediately secured in place by tightening the two silk sutures around the CCA and trimming off the end of the occluding suture with micro scissors (Fig. 5). In this position, the suture occludes the middle cerebral artery (MCA) origin and MCA territory becomes ischemic. Note the position of the end of the occluding suture in relation to the caudal suture occluding the CCA. This observation is useful in determining if any movement of the occluding suture occurred during the occlusion period. The wound is closed and the animal is allowed to recover during the 90-min occlusion period.

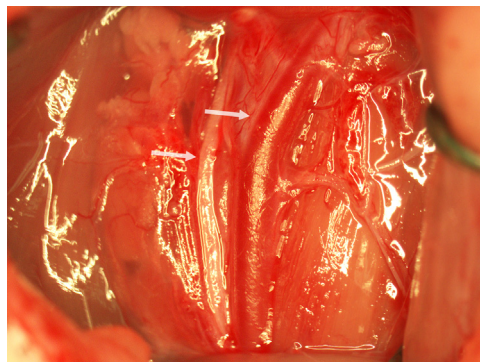


Fig. 1. The retracted surgical field showing the bifurcation of the CCA (upper arrow) and the vagus nerve (lower arrow).

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