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A blinded randomized assessment of laser Doppler flowmetry efficacy in standardizing outcome from intraluminal filament MCAO in the rat

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HIGHLIGHTS

- We evaluated the value of LDF use in rat intraluminal MCAO.
- LDF did not affect neurologic or histologic outcome.
- LDF decreased coefficient of variation for histologic but not neurologic outcome.
- LDF may allow use of fewer animals when homogeneity of infarct size is important.
- Laser Doppler use during intraluminal MCAO should be optional for surgeons.

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ABSTRACT

Background: Laser Doppler flowmetry (LDF) is widely used for estimating cerebral blood flow changes during intraluminal middle cerebral artery occlusion (MCAO). No investigation has systematically examined LDF efficacy in standardizing outcome. We examined MCAO histologic and behavioral outcome as a function of LDF measurement.
Materials and methods: Rats were subjected to 90 min MCAO by 4 surgeons having different levels of MCAO surgical experience. LDF was measured in all rats during ischemia. By random assignment, LDF values were (Assisted) or were not (Blinded) made available to each surgeon during MCAO (n = 12–17 per group). Neurologic and histologic outcomes were measured 7 days post-MCAO. A second study examined LDF effects on 1-day post-MCAO outcome.
Results: Pooled across surgeons, intra-ischemic %LDF change (P = 0.12), neurologic scores (Assisted *vs.* Blinded = 14 ± 6 *vs.* 13 ± 7, P = 0.61, mean ± standard deviation) and cerebral infarct volume (162 ± 63 mm^3 *vs.* 143 ± 86 mm^3, P = 0.24) were not different between groups. Only for one surgeon (novice) did LDF use alter infarct volume (145 ± 28 mm^3 *vs.* 98 ± 61 mm^3, P = 0.03). LDF use decreased infarct volume coefficient of variation (COV) by 35% (P = 0.02), but had no effect on neurologic score COV.
Comparison with existing methods: We compared intraluminal MCAO outcome as a function of LDF use.
Conclusions: LDF measurement altered neither neurologic nor histologic MCAO outcome. LDF did not decrease neurologic deficit COV, but did decrease infarct volume COV. LDF may allow use of fewer animals if infarct volume is the primary dependent variable, but is unlikely to impact requisite sample sizes if neurologic function is of primary interest.

Abbreviations: MCAO, middle cerebral artery occlusion; MCA, middle cerebral artery; LDF, laser Doppler flowmetry; CBF, cerebral blood flow; COV, coefficient of variation; SAH, subarachnoid hemorrhage.

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1. Introduction

Rat models of cerebral ischemia are important for increasing our understanding stroke pathophysiology and investigation of potential therapeutics. In these models, temporary or permanent middle cerebral artery occlusion (MCAO) is widely employed.

The middle cerebral artery (MCA) can be occluded through either transcranial exposure or intraluminal filament insertion. The transcranial approach allows direct MCA visualization assuring occlusion, but this requires sophisticated microsurgical skills. Intraluminal MCAO is instead achieved by introduction of a filament into the internal carotid artery, which is advanced to occlude the MCA (Koizumi et al., 1986; Longa et al., 1989; Memezawa et al., 1992). This requires only a ventral cervical skin incision and isolation of the major extracranial internal and external carotid arteries. Thus, less surgical expertise is required. The intraluminal approach also obviates interruption of muscles of mastication and periocular edema often associated with transcranial approaches. Further, the intraluminal ischemic insult can be performed largely in the absence of anesthesia abrogating that potential confound in defining ischemic outcome (Sakai et al., 2007). Because insertion of the filament is blind, proper positioning of the filament so as to occlude the MCA is uncertain. Inconsistent occlusion may cause a decreased or variable ischemic insult. Advancing the filament too far can cause vessel perforation and subarachnoid hemorrhage (SAH).

Use of continuous laser Doppler flowmetry (LDF) to obtain a consistent magnitude of cerebral blood flow (CBF) decrease during intraluminal occlusion and reperfusion has been advocated to confirm proper filament placement (Hungerhuber et al., 2006; Schmid-Elsaesser et al., 1998; Soriano et al., 1997; Woitzik and Schilling, 2002). However, no investigation has systematically examined LDF effectiveness in standardizing the severity of ischemic injury. It is important to define this because the LDF system is expensive and requires an additional scalp incision and creation of a skull burr hole in animals potentially destined for recovery analysis.

This study was designed to test the hypothesis that LDF use does not alter behavioral or histologic outcome from intraluminal MCAO. Surgeons performed MCAO with and without LDF values obtained during intraluminal MCAO. Neurologic and histologic outcome were compared measured 7 days post-MCAO. As a secondary measure, we considered the effect of LDF on outcome variability. An additional study was performed to assess LDF effects on outcome in a hyperacute setting of 1-day recovery.

2. Materials and methods

2.1. Surgical procedures

This study was approved by the Duke University Animal Care and Use Committee, Durham, NC.

Male Wister rats (Harlan Sprague Dawley, Inc. Indianapolis, IN) weighing 250–300 g (age 10–12 weeks) were allowed to access water but fasted for 12 h to standardize glycemic state. Anesthesia was induced with 5% isoflurane in oxygen. Following tracheal intubation, the lungs were mechanically ventilated to maintain normocapnia. The inspired isoflurane concentration was adjusted to 1.5% in 40% oxygen/balance nitrogen to maintain anesthesia. A 22-gauge needle thermistor was percutaneously placed adjacent to the skull beneath the temporalis. Pericranial temperature was controlled at $37.5 \pm 0.2\,^{\circ}C$ during anesthesia by surface heating or cooling. The tail artery was cannulated and used to monitor mean arterial pressure and sample blood. Heparin (50 IU) was given at the time of arterial cannulation to prevent thrombosis.

The rat was positioned prone. *Via* scalp incision, a burr hole (3 mm diameter) was made in the right temporal bone (1 mm posterior and 5 mm lateral to bregma) to expose the dura. A small plastic cylinder (1/8 inch internal diameter and 7 mm deep) was glued to the burr hole. The rat was turned supine and the LDF probe (TSI Laserflo, BPM 403A, TSI Inc., Shoreview MN) was inserted into the cylinder and opposed to the dura.

Rats were prepared for MCAO as previously described (Koizumi et al., 1986; Longa et al., 1989; Memezawa et al., 1992) with modification. A midline ventral neck incision was made and the right common carotid artery identified. The external carotid artery was isolated and the occipital, superior thyroid, and external maxillary arteries were ligated and divided. The internal carotid artery was dissected distally until the origin of the pterygopalatine artery was visualized. A 20-min interval was allowed for physiologic stabilization. Arterial carbon dioxide and oxygen tensions, arterial pH, blood glucose, and hematocrit were measured 15 min pre-MCAO, 45 min post-MCAO onset, and 15 min after reperfusion.

Rats were assigned to either LDF assisted (Assisted) or blinded (Blinded) groups by computerized randomization. In the Assisted group, LDF values were continuously available to the surgeon throughout the procedure. In the Blinded group, the surgeon was not allowed to see flow velocity values displayed on the LDF monitor at any time.

To initiate MCAO, a standardized 0.25-mm diameter nylon monofilament, prepared with a silicone tip, was inserted into the external carotid artery stump and advanced into the internal carotid artery. In the Assisted group, at least a 60% LDF decrease from the pre-ischemic baseline value was considered to be sufficient ischemia. The surgeons were allowed to manipulate the filament during the ischemic insult so as to maintain LDF values below this target. In the Blinded group, the filament was advanced until a resistance was felt (typically 18–19 mm distal to the carotid bifurcation). In both groups, LDF values were continuously monitored and recorded every minute from 15 min prior to ischemia until 15 min after reperfusion onset.

After 90 min MCAO, the filament was removed to allow reperfusion and the external carotid stump was ligated. The tail artery catheter was removed. Wounds were infiltrated with 0.25% bupivacaine and closed with suture. Isoflurane was discontinued. Upon recovery of the righting reflex, the trachea was extubated. The rats were placed in an acrylic chamber with enriched oxygen for 1 h and then returned to their cages.

2.2. Neurologic assessment

A screening neurologic examination was performed 1 h after reperfusion by use of a 4-point scoring system (Bederson et al., 1986). After 1 or 7 days recovery, the rats underwent a neurologic examination to evaluate sensorimotor function. Four different functions were evaluated (general status, simple motor deficit, complex motor deficit, and sensory deficit) by use of a previously described neurologic scoring system (Yokoo et al., 2004) that incorporates the major elements of the Bederson and Garcia scoring systems (Bederson et al., 1986; Garcia et al., 1995). Total possible scores ranged from 0 (no deficit) to 48 (maximal deficit). Values from this scoring system correlate with total infarct volume in rats (Sakai et al., 2007; Sheng et al., 2012; Yokoo et al., 2004). Observers were blinded to group assignment and were experienced in performing this scoring system. For each surgeon, a single observer scored all animals to minimize variability in application of the scoring system between groups.