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Abstract. Rodent middle cerebral artery occlusion (MCAO) model is commonly used in stroke research. Creating a stable infarct volume has always been challenging for technicians due to the variances of animal anatomy and surgical operations. The depth of filament suture advancement strongly influences the infarct volume as well. We investigated the cerebral blood flow (CBF) changes in the affected cortex using laser speckle contrast imaging when advancing suture during MCAO surgery. The relative CBF drop area (CBF_{50}, i.e., the percentage area with CBF less than 50% of the baseline) showed an increase from 20.9% to 69.1% when the insertion depth increased from 1.6 to 1.8 cm. Using the real-time CBF_{50} marker to guide suture insertion during the surgery, our animal experiments showed that intraoperative CBF-guided surgery could significantly improve the stability of MCAO with a more consistent infarct volume and less mortality. [DOI: 10.1117/1.JBO.20.9.096012]

Keywords: stroke model; middle cerebral artery occlusion; animal surgery; cerebral blood flow; laser speckle contrast imaging.

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1 Introduction

Ischemic stroke is a leading cause of disability and mortality worldwide. An animal model of middle cerebral artery occlusion (MCAO) has been considered as an effective alternative to study the mechanisms and therapy of ischemic stroke.[1] So far, the intraluminal filament rodent MCAO model introduced by Koizumi et al.[2] and modified by Longa et al.[3] has been the most widely used method for its advantages of no craniotomy and the applicability for inducing reperfusion.[4]

To ensure a successful and stable MCAO, the depth of filament insertion from the bifurcation of the common carotid artery (CCA) toward the middle cerebral artery (MCA) should be carefully controlled.[5] However, in practice, the variance of infarct volume induced by MCAO is high due to the variability in animal weight, brain vascular anatomy, suture material, filament insertion depth, and surgical operations.[6–10] Nevertheless, the conventional intraluminal filament MCAO model often simply calibrates suture with the same insertion depth, which ignores the aforementioned variability across animals. Furthermore, the success of occlusion can be confirmed mainly by postoperative methods including behavior tests, imaging technologies, e.g., computed tomography (CT), magnetic resonance imaging (MRI), and/or histologic analysis. So far, the only intraoperative method for confirming the success of MCAO is to use the laser Doppler flowmetry (LDF) to check the cerebral blood flow (CBF) drop in MCA immediately after the surgery. However, due to the high sensitivity to motion and the lack in spatial resolution, LDF in most cases could only be used as a postsurgical confirmation.[11]

Our previous work has reported the success of the two-dimensional technique of laser speckle contrast imaging (LSCI) of CBF as an early and real-time predictor of ischemic lesion after stroke in MCAO surgery.[12] Compared with LDF, LSCI provides full-field CBF information with high spatial and temporal resolution.[13] Experiments indicated that the area with more than 50% CBF drop at the first minute immediately after the occlusion had significant prognostic value in lesion volume 24 h after stroke.[12] With these inspirations, we propose to use real-time LSCI to guide the MCAO surgery so as to adjust the filament insertion depth accordingly to improve the consistency of the lesion. The ultimate goal of our work is to improve the stability of the intraluminal MCAO model and provide a CBF-guide strategy for neurovascular surgery through intraoperative LSCI.

2 Materials and Methods

The experimental protocols in this study were approved by the Animal Care and Use Committee of Med-X Research Institute of Shanghai Jiao Tong University.

2.1 *Animal Preparation*

Thirty four adult male Sprague-Dawley rats (260 to 300 g, Shanghai Slac Laboratory Animal Co., Ltd., Shanghai, China) were used for this study. Each rat was anesthetized with isoflurane (5% initial and 2.0% to 2.5% for maintaining, Abbott Laboratories Inc., Shanghai, China) induced with a mask (Model No. 68602, RWD Life Science Inc. Shenzhen, China) connected to an isoflurane vaporizer (Midmark Co., Dayton, Ohio) during the experiment. The rectal temperature was maintained at 37.0°C ± 0.5°C with a heating pad (the DC temperature

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control system, FHC Inc., Bowdoin, Maine). All procedures were performed under standard sterile precautions. The rat was placed in a stereotaxic frame (Leica Microsystems Inc., Buffalo Grove, Illinois). A midline incision was made on the scalp, and the tissues over bones were cleaned with a blade. A cranial window centered at 3.5-mm posterior to the bregma over the cortex was thinned by a high-speed dental drill (Fine Science Tools Inc., North Vancouver, Canada) with ∅1.4-mm steel burr until the cortical vessels were clearly visible. Saline was used to cool down the skull during thinning. Then a cylinder base was fixed onto the skull over the cranial window with reinforced glass ionomer cement (Dental Materials Factory of Shanghai Medical Instruments Co., Shanghai, China) as we did in the previous study.[12] After the cement hardened, the rat was placed in a supine position and a full-field LSCI CBF imager (RatCap-3, Dolphin BioTech Ltd., Shanghai, China) was connected to the cylinder base to acquire intraoperative real-time cortical CBF images.[12,13] During the experiment, the rat skull was illuminated by a 780-nm laser diode (L780P010, Thorlabs Inc., Newton, New Jersey) and the laser speckle images (640×640 pixels) were acquired at 40 fps.

2.2 *Real Time Laser Speckle Contrast Imaging*

The theories and technical details of LSCI have been documented in the literature.[14,15] In this study, the contrast value K was calculated using temporal laser speckle contrast analysis with 320 frames after registration.[16] K^2 is inversely proportional to the CBF speed

$$K^2 = \frac{\sigma_s^2}{\langle I \rangle^2} = \beta \left\{ \frac{\tau_c}{T} + \frac{\tau_c^2}{2T^2} \left[\exp\left(-\frac{2T}{\tau_c} \right) - 1 \right] \right\}, \tag{1}$$

where T is the exposure time of the CCD and the autocorrelation time τ_c is inversely proportional to the CBF velocity. β is a constant accounting for the loss of correlation. All data were analyzed with MATLAB® software (Ver. R2013b, Mathworks Inc., Natick, Massachusetts).[17]

2.3 *Middle Cerebral Artery Occlusion Surgery*

A neck midline incision was made, and the soft tissues were pulled apart to expose the vessels. The right (CCA) and the right external carotid artery (ECA) were dissected and ligated using 4-0 suture (Jinhuan Medical Products Co., Ltd., Shanghai, China). Then the right internal carotid artery (ICA) was separated and occluded temporally by a microvascular clip. A silk suture was loosely tied around the CCA close to the clip, which would be fastened to secure the inserted suture after the occlusion was made. A 4-0 endovascular suture with 5-mm silicone coating length (Sunbio Biotech Co., Ltd., Beijing, China) was advanced via the incision into the right CCA toward the right ICA. The clip was then released and then the suture was advanced up to a premarked insertion depth.[5,18] In the LSCI guided group, the suture insertion was adjusted to meet the criteria of CBF changes (see Sec. 2.6). After the MCAO, the remaining suture was cut and the neck incision was closed. Laser speckle images were continuously recorded starting from the beginning of the surgery up to 30 min after the occlusion (Fig. 1).

2.4 *Infract Volume Calculation*

Twenty-four hours after MCAO, the rats were euthanized and the brains were isolated and sectioned coronally into five slices (thickness: 3 mm) with brain matrices (Model No. 68710, RWD Life Science Co., Ltd,). All brain slices were stained with 2,3,5-triphenyltetrazolium chloride (TTC, Sigma-Aldrich Co. LLC, St. Louis, Missouri) at 37°C for 10 min in a dark chamber. The infarct volume was quantitated by ImageJ software (Ver. 1.44, National Institutes of Health).[19] The infarct area for each slice was measured by subtracting the non-ischemic area of ipsilateral hemisphere from that of the contralateral hemisphere. The infarct volume was calculated as the summation of all the slice infract area multiplied by the slice thickness.

2.5 *Data Processing*

We investigated the full-field intraoperative CBF variations during the MCAO model. Three regions of interest (ROIs) were selected for quantitative CBF analysis, including the vein in the right hemisphere (RV), the artery in the right hemisphere (RA), and the capillary bed in the somatosensory area of the right hemisphere (RC) [Fig. 1(b)]. In the CBF-guided MCAO model, we used the ratio of the cortical area with more than 50% CBF (i.e., CBF_{50}) drop as the criterion for its early prognostic value.[12] At each recording time, the relative CBF_{50} reduction area (R_{50}) is defined

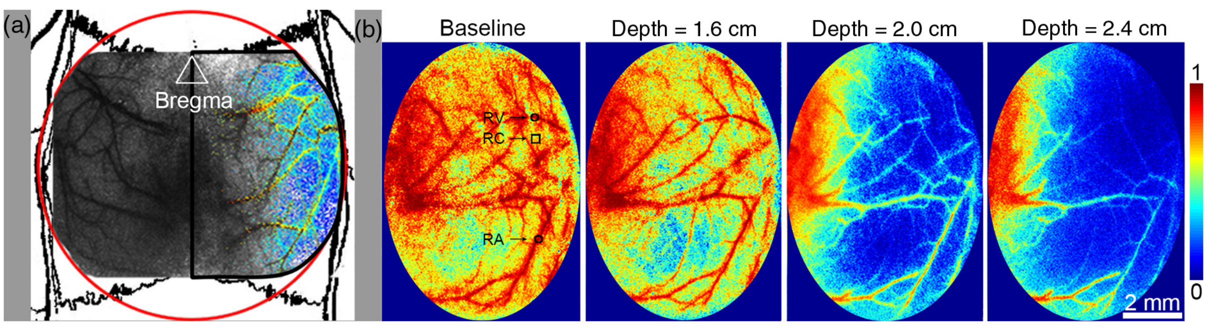

Fig. 1 Schematic of the cranial window for cerebral blood flow (CBF) imaging during the surgery. (a) Laser speckle contrast image in gray scale acquired before surgery overlaid with post-occlusion CBF reduction area in pseudo-color. Black box in the figure, region of right hemisphere. (b) The right hemispheric CBF images recorded at baseline level and at various suture insertion depths during the middle cerebral artery occlusion surgery. Three regions of interest (ROIs) are selected in the pseudo-color image: RV, the vein in the right hemisphere; RA, the artery in the right hemisphere; and RC, the capillary bed in the somatosensory area of the right hemisphere.

$$R_{50}(\%) = \frac{\text{Pixles of CBF}_{50}}{N_{\text{pixel}}} \times 100\%, \tag{2}$$

where N_{pixel} is the number of all pixels in an ∼4(horizontal) × 7(vertical) mm^2 right hemisphere cranial window [centered at AP, −3.5 mm; ML, 2 mm, as shown in Fig. 1(a)] over the right hemisphere. An illustration of CBF_{50} right upon MCA occlusion is shown in Fig. 1(a), where the CBF_{50} reduction area is presented in pseudocolor overlying the laser speckle contrast image.

2.6 *Experiment Design*

2.6.1 *Experiment A*

We monitored the CBF evolution while manipulating the suture insertion depth during the standard MCAO surgery. Eight rats were used in this experiment. During the surgery, the CBF was recorded for the suture insertion starting from 1.4 up to 2.4 cm with a step of 0.2 cm. The selection of this insertion range is based on the rat vascular anatomical features. The corresponding CBF images for a representative rat were shown in Fig. 1(b).

2.6.2 *Experiment B*

We compared the conventional MCAO model and the CBF-guided MCAO model. Twenty-six rats were randomly divided into two groups. In the conventional MCAO group ($N = 16$), the suture was advanced up to 1.8 cm from the bifurcation of right CCA, which followed the standard protocols used in most previous literature. In the CBF-guided MCAO group ($N = 10$), the CBF_{50} reduction area was monitored continuously and the insertion of the suture immediately ceased when $R_{50} = 50\%$ was reached. This criterion was set according to the correlation information between the CBF_{50} reduction area and lesion volume obtained from Experiment A (see Sec. 3.1). Twenty-four hours after the MCAO, infarct volume was calculated after euthanizing the rats and removing the brains.

2.7 *Statistics Analysis*

Statistical comparison of CBF during the entire MCAO surgery was carried out using a two-tailed *t*-test with SPSS (Ver. 21.0, SPSS Inc., Chicago, Illinois). All data are presented as the mean ± SD. Statistical significance was assumed when $P < 0.05$.

3 Results

3.1 *Spatiotemporal Changes of Cerebral Blood Flow with Varying Suture Insertion*

First, we investigated the spatiotemporal changes of CBF while manipulating the suture insertion depth during MCAO surgery in experiment A. Three ROIs representing vein, artery, and capillary areas, respectively, were selected for analysis, as shown in Fig. 1(b). In addition, the N_{pixel} area was highlighted and R_{50} was estimated when adjusting the insertion depth. Figure 2(a) shows the average relative CBF in each ROI when the suture insertion depth increased from 1.4 to 2.4 cm ($N = 8$). Overall, the relative CBF in all ROIs decreased when advancing the suture into the vessel. It should be noted that the CBF drop in vein was less than that in artery or capillary. Apparently, the most significant CBF drop occurred when suture was advanced from 1.6 to 1.8 cm, e.g., the relative CBF dropped from 61.7% ± 7.2% to 29.8% ± 2.8% in artery, from 62.3% ± 5.1% to 35.4% ± 4.0% in vein, and from 62.9% ± 5.6% to 30.1% ± 2.9% in capillary bed, respectively. CBF became stable when the insertion depth was over 1.8 cm. Figure 2(b) shows the changes of R_{50}, which concordantly indicates a significant increase from 20.9% ± 6.4% to 69.1% ± 9.9% while the suture insertion depth increased from 1.6 to 1.8 cm. Therefore, 1.6 to 1.8 cm is the critical range of suture advancement in this MCAO surgery, which is consistent with the fact that 1.8 cm has been recommended for suture insertion by conventional MCAO surgery protocols. It is noted in Fig. 2(b) that R_{50} increased approximately from 30% to 70% in the critical suture insertion range, we, therefore, used the medium value (50%) as the criterion to guide the MCAO surgery in experiment B.

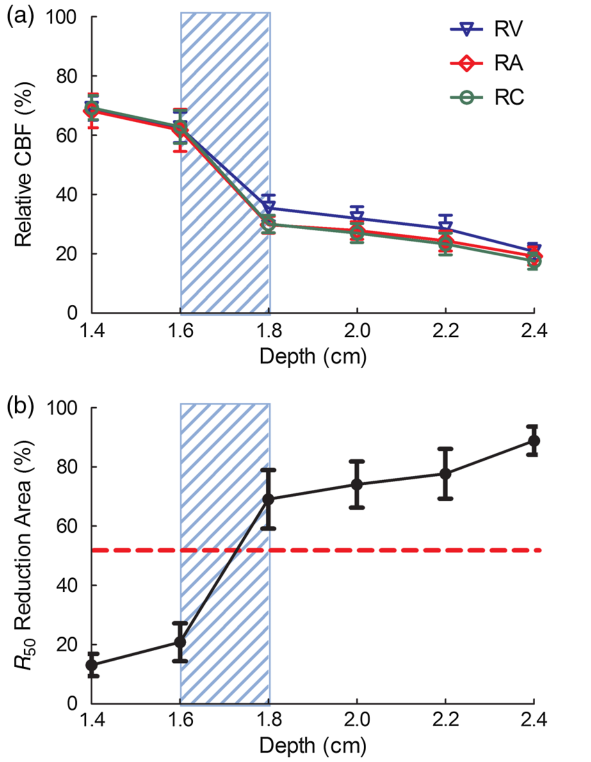

Fig. 2 (a) The variation of relative CBF in different ROIs during the increase of suture insertion depth ($N = 8$); (b) the relative CBF_{50} reduction area (R_{50}) during the increase of suture insertion depth ($N = 8$).

3.2 *Image Guided Middle Cerebral Artery Occlusion Versus Conventional Middle Cerebral Artery Occlusion Surgery*

In the conventional MCAO group ($N = 16$) without CBF guidance, two rats died within 24 h after the surgery. Therefore, we further analyzed the data for the remaining 14 rats in this group. When the suture was advanced up to 1.8 cm from the bifurcation of the right CCA, we stopped the suture insertion and the corresponding R_{50} of each rat is shown in Fig. 3(b). Two rats were found with no visible lesion in infract volume, and with R_{50} equal to about 0. The overall success rate of conventional MCAO was $12/16 = 75\%$. The average infarct area in the conventional MCAO group was $0.417 \pm 0.217\ \text{cm}^3$ ($N = 14$). The variance of infarct volume over mean value was 51.87%

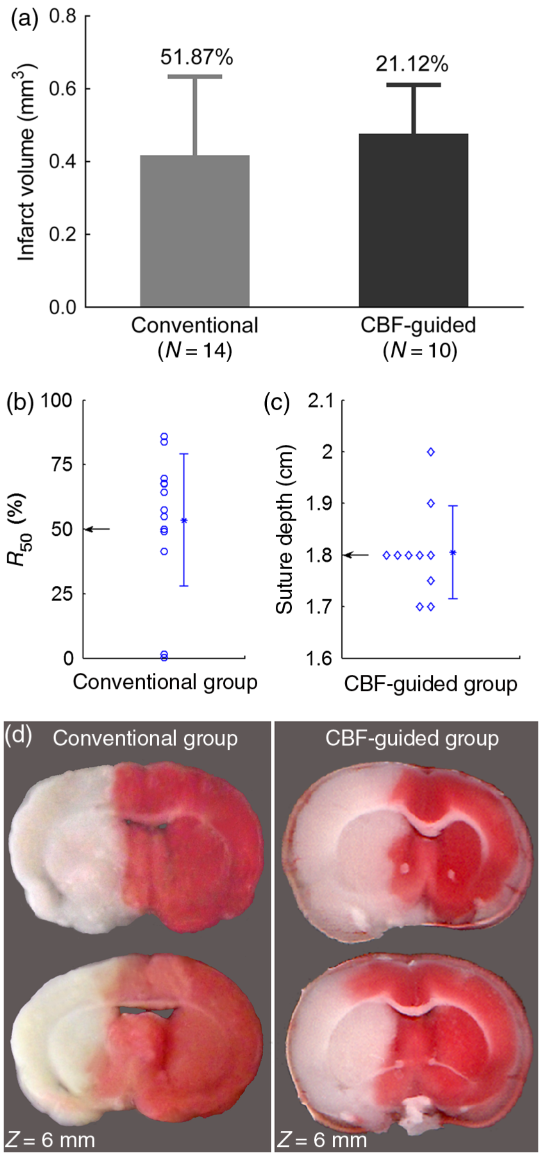

Fig. 3 (a) Comparison of infarct volume between the conventional and the CBF-guided groups. The variance is expressed in percentage. (b) R_{50} of different rats for suture insertion at 1.8 cm in conventional group. (c) The insertion depths of different rats at $R_{50} = 50\%$ in CBF-guided group. (d) 2,3,5-triphenyltetrazolium chloride stained brain slices of representative rats, 6 mm distal from the frontal pole, from different groups in experiment B.

[Fig. 3(a)]. In the CBF-guided MCAO group, we immediately stopped the suture insertion as long as we observed that R_{50} increased to 50%. The insertion depths of different rats are shown in Fig. 3(c). The stroke modeling was successful in all rats (100%) and the infarct area was $0.477 \pm 0.134\ \mathrm{cm}^3$, with a ratio of variance over mean equal to 21.12% [Fig. 3(a)]. In addition, we performed F-test analysis (one-sided test) using MATLAB® software for statistical comparison of variance between the conventional and the CBF-guided groups. The results showed that the conventional group had a significantly greater variance than the CBF-guided group (51.87% versus 21.12%, $P < 0.05$). In Fig. 3(d), TTC-stained brain slices of representative rats from different groups in experiment B are shown. Therefore, the CBF-guided surgery improved the infarct volume consistency and success rate of stroke.

4 Discussions

A stable rodent model of MCAO is of great importance in stroke studies. Though the intraluminal MCAO model has been the most common technique in experimental stroke studies, high variance of infarct volume has limited its applications. Excessive insertion of suture could increase the risk of subarachnoid hemorrhage and thus bring higher mortality rates; while insufficient insertion may not completely occlude the MCA and thus likely lead to the failure of the model. Except for the anatomic variance in animals, the success rate of the surgery strongly depends on the skills of the technician. Compared with the current "blind" surgery, the new CBF-guided MCAO model offers a "closed-loop" surgical guidance for the technician and thus improves the stability as well as the success rate.

Although there are other techniques, including magnetic resonance angiography, positron emission tomography, MRI, CT perfusion, that can be used to confirm the success of MCAO,[20,21] the poor temporal resolution hampers them from application in intraoperative settings. Although LDF has been widely used to monitor the CBF changes and confirm the success of MCAO during the surgery, it offers very limited prognostic information to guide the suture insertion due to its lack of consistency.[5,22] For example, the infarct volumes could vary a lot even though LDF showed similar CBF reduction in MCAO surgery.[23,24]

One limitation of the CBF-guided MCAO modeling is the invasiveness introduced by creating the imaging window for LSCI, though the cranial window still keeps the cerebral system intact. It should be noted that noninvasiveness could be possible for the CBF-guided MCAO model in mice, in which the skull remains intact for LSCI.

5 Conclusion

In this study, we confirmed the prognostic value of early CBF in different types of vessels and demonstrated dynamic changes of CBF during MCAO surgery when manipulating the suture insertion. We proposed the relative CBF_{50} ($R_{50} = 50\%$ in this study) as the criterion to guide the suture advancement to ensure a successful MCAO. Our experiments showed that the CBF-guided MCAO significantly reduced the variance of stroke lesion and increased the success rate of this model.

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