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Imaging the Early Cerebral Blood Flow Changes in Rat Middle Cerebral Artery Occlusion Stroke Model

Shuping Zhu, Yao Li[*], Hongyang Lu, Hangdao Li and Shanbao Tong, *Senior Member, IEEE*

Abstract—**Intraluminal middle cerebral artery occlusion (MCAO) model in rats has been widely used to mimic human ischemic stroke and serves as an indispensable tool in the stroke research field. One limitation of this model is its high variability in infarct volume. The cerebral blood flow (CBF) information after cerebrovascular occlusion may reflect the availability of collateral circulation, which serves as a key factor for brain infarct volume. Laser speckle contrast imaging (LSCI) is a valuable tool for full-field imaging of CBF with high spatial and temporal resolution. In this paper, we investigated the spatio-temporal changes of CBF in rat MCAO stroke model using our self-developed real-time LSCI system. CBF images of adult male Sprague Dawley rats (n=13) were recorded before surgery, during first 1.5 hours after surgery, and 24 hours after stroke. We compared the CBF changes of different functional vessels during this period. In the ipsilateral hemisphere, CBF of veins and arteries both decreased as expected, while CBF of veins increased after occlusion in the contralateral hemisphere. Moreover, we found a linear correlation between early-stage CBF after occlusion and brain infarct volume, which can be utilized for surgery guidance to improve the uniformity of rat MCAO stroke models.**

I. INTRODUCTION

Middle cerebral artery occlusion (MCAO) model in the rat has been in use since 1975 [1]. This experimental focal cerebral ischemia model has been developed to mimic human ischemic stroke and serves as an indispensable tool in the stroke research field [2]. Among a variety of methods for making MCAO models, the intraluminal filament technique has been mostly used due to its relatively noninvasive and reversible features [3-5]. However, one major limitation of the intraluminal filament model is the high variability in infarct volume arising from various factors such as animal brain vascular anatomy and weight, suture material, and sites of occlusion, etc. [6-8]. Thus an effective observation method is in need so as to evaluate the severity of ischemic brain injury during intraluminal filament MCAO surgery, which is directly related to the brain infarct volume.

It has been shown that cerebral blood flow (CBF) reduction after cerebrovascular occlusion may reflect the availability of collateral circulation, which serves as a key contribution for brain infarct volume [9]. In the past two decades, laser Doppler is one of the widely employed techniques to monitor the CBF changes during MCAO surgery to help determine whether the stroke experiment is successful. To ensure a successful occlusion, a drop to 20% to 30% of baseline level in blood flow on the surface of the ipsilateral dorsolateral cortex would be observed by laser Doppler flowmetry (LDF). However, CBF information obtained by LDF is constrained to very limited locations. The infarct volumes still vary a lot even though the surgical operations are performed with the assistance of LDF [10]. Thus it is valuable to obtain CBF information in a more comprehensive way during the MCAO surgery to improve uniformity of the brain infarct volume.

Laser speckle contrast imaging (LSCI) technique is an optical functional imaging methodology widely used for monitoring the spatio-temporal changes of CBF. The velocity of blood flow is approximated by calculating the speckle contrast [11]. Compared with LDF, LSCI can obtain full-field information about CBF with high spatial and temporal resolution in a less expensive way. Utilizing our self-developed laser speckle imaging system [12], we are able to obtain real-time CBF information in great details during and immediately after the rat MCAO surgery.

In this study, we aim to find out the temporal changes of CBF during and immediately after intraluminal rat MCAO model and investigate its correlation with brain infarct volume 24 hours after the stroke.

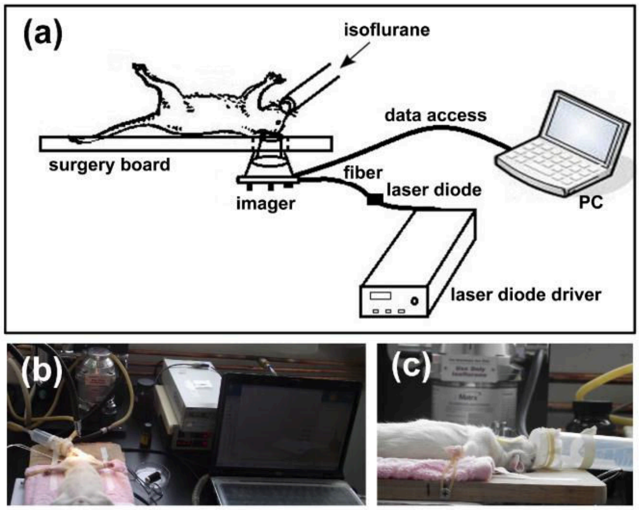

Figure 1. Real-time laser speckle imaging system during surgery in schematic drawing (a) and photographs (b, c).

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S. Zhu, H. Lu, H. Li and S. Tong are with School of Biomedical Engineering and Med-X Research Institute, Shanghai Jiao Tong University, Shanghai 200030, P. R. China.

Y. Li is with School of Biomedical Engineering and Med-X Research Institute, Shanghai Jiao Tong University, Shanghai 200030, P. R. China. (phone: +86-21-62933086; fax: +86-21-34204717; yaoli118@gmail.com).

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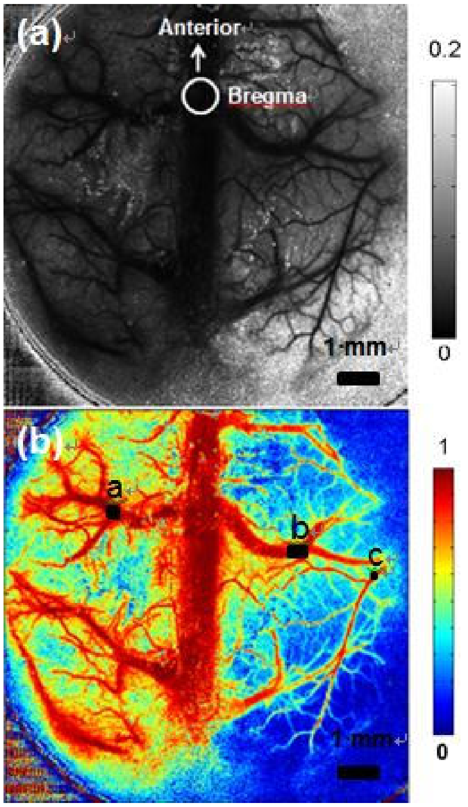

Figure 2. Laser speckle contrast image acquired after filament insertion (a) and its corresponding pseudo-color image with 3 ROIs: 2.4 mm to the middle line in the ipsilateral vein, 2.4 mm to the middle line in the contralateral vein and 5.1 mm to the middle line in the ipsilateral artery (b).

II. MATERIALS AND METHODS

A. *Animal preparation*

Adult male Sprague Dawley rats (n=13), weighing 265 to 295 g (Slac, China), were used in the experiments. Rats were anesthetized with 2.5% isoflurane, along with 20% oxygen and 80% nitrogen induced through a face mask during the experiment. The protocol used in this study has been approved by the Animal Care and Use Committee of Shanghai Jiao Tong University.

B. *Surgical procedure & Laser speckle data acquisition*

The rats were mounted on a stereotaxic frame and the skull was thinned to transparency. A miniature laser speckle imager (diameter: 15 mm) was placed on the skull and fixed with glass ionomer cement. Subsequently, the rats were transferred to the surgery table and fixed in a supine position. A laser diode (780 nm; 10 mW; L780P010, Thorlabs, USA) powered by a driver module (LDC220C, Thorlabs, USA) was used to illuminate the rat skull with a beam expanded through a collimating lens (Fig. 1). Raw laser speckle data (50 frames per second) were acquired as baseline before MCAO surgery was started. Right common carotid artery (CCA), external carotid artery and internal carotid artery were dissected. A monofilament nylon suture (length: 4.0 cm; diameter: 0.36 mm), with its 5-mm distal segment coated with silicone (BEIJING SUNBIO BIOTECH CO., LTD, China), was advanced carefully up to 18 mm into the middle cerebral artery (MCA) from the CCA junction. Laser speckle images were acquired from the completion of filament insertion to 1.5 hours afterwards, and at 24 hours after MCA occlusion. The rats were sacrificed 24 hours after surgery and brains were removed quickly, sectioned coronally into five 3-mm-thick slices, stained with 4% solution of 2,3,5-triphenyltetrazolium chloride (TTC) at 37℃ and then photographed. Infarction volume was calculated using ImageJ software (National Institutes of Health, USA) [13].

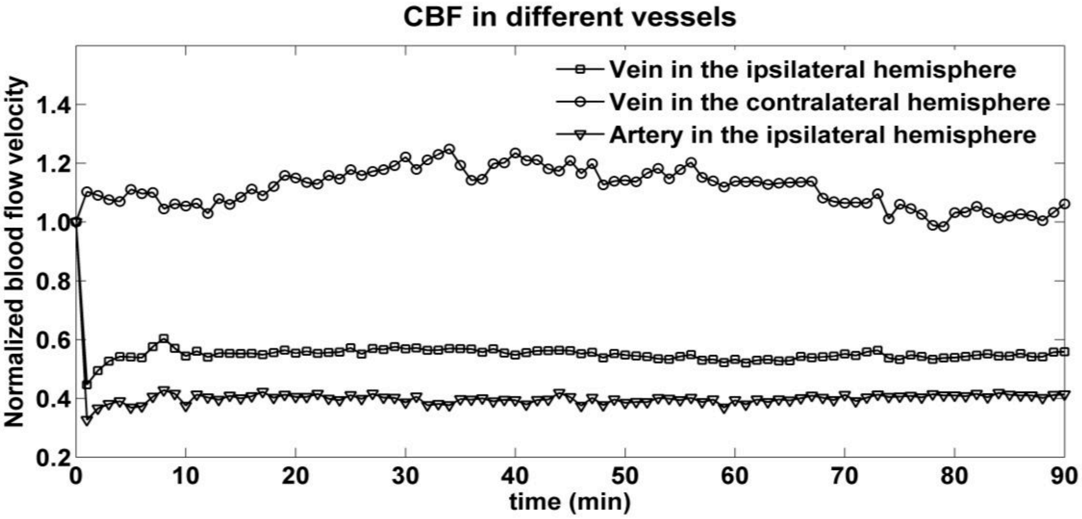

Figure 3. CBF in different vessels during 90 minutes immediately after the completion of filament insertion in MCAO stroke surgery (n=13).

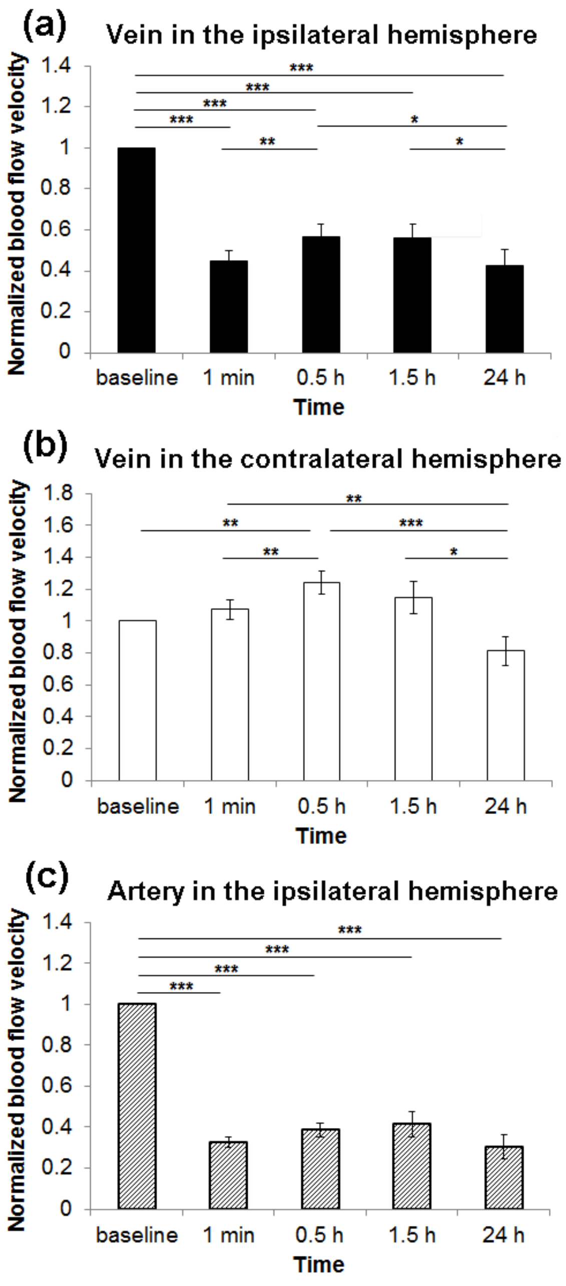

Figure 4. Blood flow velocity changes of baseline, 1 minute, 0.5 hour, 1.5 hour and 24 hours after MCAO in the corresponding vessels (n = 13).

C. Data analysis

According to the temporal laser speckle contrast analysis (tLASCA) theory [14-16], blood flow velocity is related to contrast value K which is defined as the ratio of the standard deviation to the mean intensity in the speckle pattern:

$$K = \frac{\sigma}{\langle I \rangle} \le 1 .$$

And K is related to the correlation time τ_c as:

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

where T is the exposure time and τ_c is inversely proportional to the blood flow velocity[17]. Therefore, K^2 is inversely proportional to the blood flow velocity[16].

The raw speckle images (640×640 pixels) were computed with tLASCA method to obtain the contrast image of CBF (Fig. 2). Each image was registered to reduce the disturbances due to respiration and heart beating of rats. Three regions of interest (ROIs; 2.4 mm to the middle line in the ipsilateral vein, 2.4 mm to the middle line in the contralateral vein and 5.1 mm to the middle line in the ipsilateral artery; Fig. 2) from different functional vessels in both ipsilateral and contralateral hemispheres were selected and their CBF values were calculated and normalized as percentage of the baseline level.

Statistical analysis was carried out with SPSS 15.0. Significances of the differences in all different time points were analyzed with paired *t*-test.

III. RESULTS

A. Cerebral blood flow velocity variation

The CBF velocities were recorded continuously from baseline up to 90 minutes post the occlusion of MCA in the selected ROIs (Fig. 3). CBF velocity in the vein and the artery of ipsilateral hemisphere dropped to about 45% and 30% of baseline respectively immediately after the occlusion and then recovered a little and remained close to 50% and 40% of baseline. An increase of 10% in the CBF velocity of the contralateral vein was observed after the occlusion, while 1 hour later the velocity restored to baseline level gradually.

Fig. 4 lists the comparison of normalized blood flow velocity of different vessels at several time points (baseline, 1 minute, 0.5 hour, and 24 hours after MCA occlusion). CBF of the ipsilateral vein dropped to 45% of baseline at 1 minute ($p < 0.001$), then restored to 57% of baseline at 0.5 hour ($p < 0.001$), and decreased to 43% of baseline at 24 hours ($p < 0.001$). On the contrary, CBF of the contralateral vein increased 7% compared to baseline at 1 minute, then kept rising and reached 124% of baseline at 0.5 hour ($p = 0.007$).Then it went down and was 81% of baseline at 24 hours. In the ipsilateral artery, a significant drop of about 70% was observed at 1 minute compared to baseline ($p < 0.001$) and the significance remained at 0.5 hour and 24 hours ($p < 0.001$).

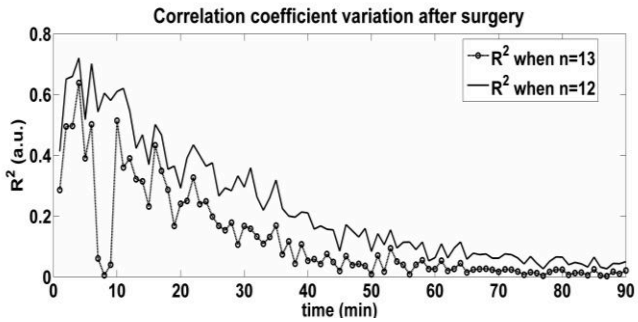

Figure 5. Variation of the correlation coefficient R^2 after surgery with and without one sample in 90 minutes after MCAO. R^2 reached the maximum value at the 4th minute.

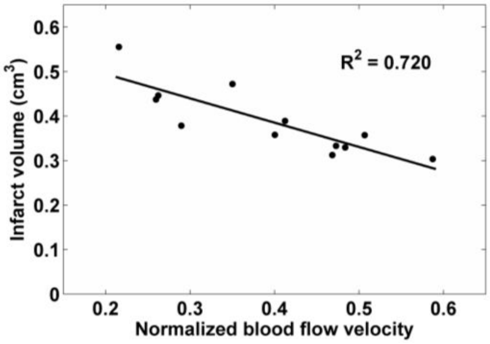

Figure 6. Correlation between the normalized blood flow velocity at the 4th minute after surgery and brain infarct volume at 24 hours after stroke (n = 12).

B. *Correlation between blood flow velocity and infarct volume*

The rat's brain was removed and stained with TTC at 24 hours after stroke surgery and the infarct volume was calculated. Moreover, the correlation coefficient R^2 between the normalized blood flow velocity and infarct volume was computed. Here we used the artery in the ipsilateral hemisphere.

We found that the correlation coefficient dropped exponentially along with post-surgery time, especially with one sample excluded. The big drop at 7 to 9 minutes for n=13 was caused by one rat sample, which we assumed to be an outlier. Fig. 5 listed the variation of correlation coefficient R^2 between CBF and brain infarct volume during the first 1.5 hours after surgery with and without the outlier sample. As seen from Fig. 5, R^2 reached the maximum value at the 4th minute after surgery ($R^2 = 0.720$). The correlation details at this time point without the outlier were shown in Fig. 6.

IV. DISCUSSIONS AND CONCLUSIONS

In this study, we investigated the full-field CBF characteristics in rat MCAO stroke model with high spatio-temporal resolution using LSCI technique. The CBF data were acquired continuously from the onset of filament occlusion till 1.5 hours afterwards. We compared the CBF changes of different functional vessels during this period. In the ipsilateral hemisphere, CBF of veins and arteries both decreased as expected, which was the consequence of ischemia after MCAO. In the contralateral hemisphere, CBF of veins increased after occlusion, which was caused by compensatory blood flow responses.

In addition, we found that the normalized CBF velocity in the branch of MCA at early stage of occlusion is linearly correlated with the infarct volume at 24 hours after stroke surgery. And the correlation coefficient reached the maximum at the 4th minute post occlusion.

Moreover, the LSI equipment we developed for real-time CBF monitoring in rat MCAO stroke model provided consistent observation conditions so as to avoid environmental and operational variations. The data were collected in exactly the same vision of each rat's skull from the very beginning of the surgery to the end, thus ensuring the accuracy of data comparison and analysis.

Our observations provide valuable information in understanding the CBF spatio-temporal changes during early stage of rat MCAO model. The correlation between CBF and brain infarct volume shows that CBF immediately after the filament occlusion can be used as a predictor of brain injury size. Moreover, LSCI may help with the guidance of surgery procedure so as to improve the uniformity of rat MCAO stroke models.

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