Early Artery Blood Flow is More Prognostic in Rodent Model of Middle Cerebral Artery Occlusion

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Abstract—**Middle cerebral artery occlusion (MCAO) by intraluminal suture is one of the most commonly used stroke models. Our previous study has indicated that the intraoperative cerebral blood flow (CBF) immediately after the stroke is prognostic for long-term permanent injury. The area of more than 50% CBF drop at the first minute after the stroke was found significantly correlated with the lesion size at 24 hours after the stroke. In order to compare the prognostic of different vessels, in this study, we further analyzed the correlation between the CBF levels in major artery, vein and the capillary bed and the lesion volume at 24 hours respectively. The results show that ipsilesional artery blood flow is of more prognostic value in MCAO lesion than the CBF in veins and capillaries.**

INTRODUCTION

Ischemic stroke has been the leading cause of disability and mortality. Stroke occurs when a cerebral vessel is either blocked or hemorrhagic. So far, animal model of middle cerebral artery occlusion (MCAO) has been concerned as an effective alternative to study the mechanisms and therapy of ischemic stroke. Among the MCAO models, the intraluminal thread model in rodents is most widely used due to its advantages, such as avoiding craniotomy and the ability of inducing transient model of MCAO [1, 2].

However, the infarct volume of the intraluminal thread model varies considerably across different rats, though the age of the rat, type and strain of suture, and location of occlusion are well controlled [3, 4]. Therefore, the early predictor of ischemic stroke has been an important topic in stroke study. So far, different methods have been introduced to detect the pathological changes during surgery and evaluate the outcome of the brain injury in real time. Among them, the cerebral blood flow (CBF) has been regarded as the most prognostic physiologic marker as it directly shows the cerebral blood perfusion [5].

For many years, laser Doppler flowmetry (LDF) has been used as a useful tool in laboratory to verify and confirm the success of the MCAO. A drop of 70~80% of baseline CBF in the dorsolateral cortex of ipsilesional hemisphere has been a standard criteria of successful stroke [6, 7]. However, because of the low spatiotemporal resolution, LDF has mostly been used as a post-surgery confirmation, but not an intraoperative monitoring. Therefore, we recently proposed to use 2-D CBF imaging, i.e. laser speckle contrast imaging (LSCI), to monitor the blood flow intraoperatively. LSCI has been used for blood flow imaging as a substitution of LDF in many researches in recently years [8]. In our recent work, we implemented a head-mounted LSCI imager for intraoperative CBF monitoring in a MCAO model, our results indicated that the area of more than 50% CBF drop at the first minute after the stroke was significantly correlated with the lesion size at 24 hours after the stroke.

In this study, we further study the prognostic value of CBF in different vessels, i.e. main artery, vein and capillary bed. We delicately select the artery, the vein and the capillary close to the ischemic core, and analyze the correlation between the CBF drop in different vessels and the lesion volume at 24 hours after the stroke.

MATERIALS AND METHODS

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

A. Animal Preparation

Fourteen adult male Sprague-Dawley rats (Shanghai Slac Laboratory Animal Co., Ltd., Shanghai, China), weighing 260~300 g, were prepared for the MCAO surgery. Each rat was anesthetized with 5% isoflurane and maintained anesthesia with 2.5% isoflurane in 2/3 N_2O and 1/3 O_2 induced with a face mask in a stereotaxic frame (Benchmark Deluxe™, MyNeurolab.com, St. Louis, Missouri) and the body temperature was maintained at 37.0 ± 0.5 °C during the whole surgery with a heating pad [9]. A midline incision was made on the scalp, and tissues over the bones were cleaned with a blade. A 7 mm × 7 mm cranial window (centered at 3.5 mm posterior to the bregma over the cortex) was thinned with a high-speed dental drill (Fine Science Tools Inc., North Vancouver, Canada) until the cortical vessels were clearly visible. All procedures were performed under standard sterile precautions. Then a cylinder base was fixed onto the skull over the cranial window with reinforced glass ionomer cements (Dental Materials Factory of Shanghai Medical Instruments Co., Shanghai, China). When cement dried, the rat was placed in supine position and a full-field cerebral blood flow imager (RatCap-3, Dolphin BioTech Ltd., Shanghai, China) was connected to the cylinder base to acquire the cortical CBF images during the experiment.

B. Imaging Procedures

The laser speckle images (640 × 640 pixels) were acquired at 40 fps (exposure time: 5 ms) with the imager over the skull illuminated by a 780-nm laser diode (L780P010, Thorlabs, USA) during the experiment [10]. Technical details of the imaging system has been described in literature [11]

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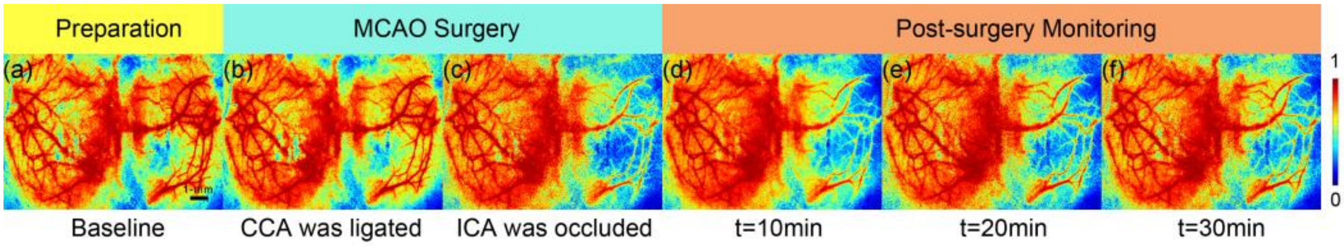

Figure 1 Schematic of the experimental protocols. (a) After the preparation of the cranial window, the CBF was recorded as baseline before the MCAO surgery. The CBF was continuously recorded during the MCAO surgery (b) and for 30 min after the middle cerebral artery was completely occluded (defined as t=0min) (c).

C. MCAO Surgery

The right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were exposed through a midline incision. Then the right CCA and ECA were ligated, and the right ICA was occluded by a curved microvascular clip temporally. A nylon suture (length: 4 mm; diameter: 0.36 mm) with 5-mm silicone coating (Beijing Sunbio Biotech Co., Ltd, Beijing, China) was advanced via a small incision in the right CCA (between the bifurcates to the right ECA and ICA and the knot in right CCA) into the right ICA [12]. After the incision was fastened and the occultation was released, the suture was advanced up to 18 mm from the bifurcation of right CCA. Finally, the remaining suture was cut and the incision was closed. Laser speckle images were recorded from the start of the surgery up to 30 minutes after the occlusion. The experimental protocols are shown in Fig. 1.

D. Infract Volume Calculation

Twenty-four hours after MCAO, rats were sacrificed, and brains were isolated and sectioned coronally into five 3-mm-thick slices in brain matrices (RWD Life Science Co., Ltd, Shenzhen, China). All brain slices were stained with 2,3,5-triphenyltetrazolium chloride (TTC, Sigma-Aldrich Co. LLC., St Louis, MO) at 37 °C for 10 minutes in a dark chamber. Infarction volume was calculated by ImageJ software (National Institutes of Health, USA) [13].

E. Data Processing

The theories and technical details of LSCI have been well documented in literature [14, 15]. Briefly, the contrast values K_s for laser speckle images were calculated every 320 frames of registered images by the temporal laser speckle contrast analysis method [16]. The squared K_s is inversely proportional to the CBF speed:

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

where T is the exposure time of the CCD and the autocorrelation time τ_c is inversely proportional to the CBF speed [15]. β is a constant parameter accounting for the loss of correlation [17]. All data were analyzed off-line with Matlab software (ver. R2013b, Mathworks Inc., Massachusetts, USA). Fig.1 shows CBF images in pseudo-color at different time during the experiment.

F. Statistics Analysis

Statistical comparison of CBF during the whole MCAO surgery was carried out using a two-way analysis of variance (ANOVA) with SPSS (ver. 21.0, SPSS Inc., Chicago, USA). All data are presented as the means ± SD. Statistical significance was assumed when $P < 0.05$.

RESULTS

A. Correlation between CBF Reduction Areas and Infarct Volume

Fig. 2(a) shows a representative laser speckle contrast image before MCAO. To study the CBF change during the stroke formation, we quantify the contrast change at each pixel at different time relative to the baseline level. The cortical area $A_t(\varepsilon)$ with a CBF drop more than a threshold (ε) at time t is then defined to describe the severity of the ischemia (e.g. $\varepsilon = 50\%$ was used in our previous work [11]). In practice, $A_t(\varepsilon)$ could be approximated with the number of pixels with relative contrast values less than ε, i.e.

$$B_t(i,j) = \begin{cases} 1, & N_t(i,j) < \varepsilon \\ 0, & \text{otherwise} \end{cases} \qquad (2)$$

where $\{B_t(i,j) = 1\}$ represents the region with CBF drop more than ε, and $N_t(i,j)$ is relative CBF. Therefore, relative CBF reduction area can be calculated as:

$$A_t(\varepsilon) = \frac{\sum B_t(i,j)}{B} \qquad (3)$$

where B is the pixel number of the whole right hemisphere. For the example in Fig. 2(b), $t = 0$ and the region of B is circled with dotted line for $\varepsilon = 40\%$. For better visualization, the image for CBF reduction region in pseudo-color is plotted and overlaid on the laser speckle contrast image.

We then computed the correlation coefficient R^2 between the CBF reduction areas $A_t(\varepsilon)$ up to 30 minutes post MCAO and infarct volume for different threshold ε ranging from 20% to 55%, as shown in Fig. 2(c). With the increase of threshold ε, R^2 rises at almost every minute after stroke. Particularly, it should be noted that R^2 was more stable after 20 minutes than that at the first 15 minutes post-surgery. For the experiments in

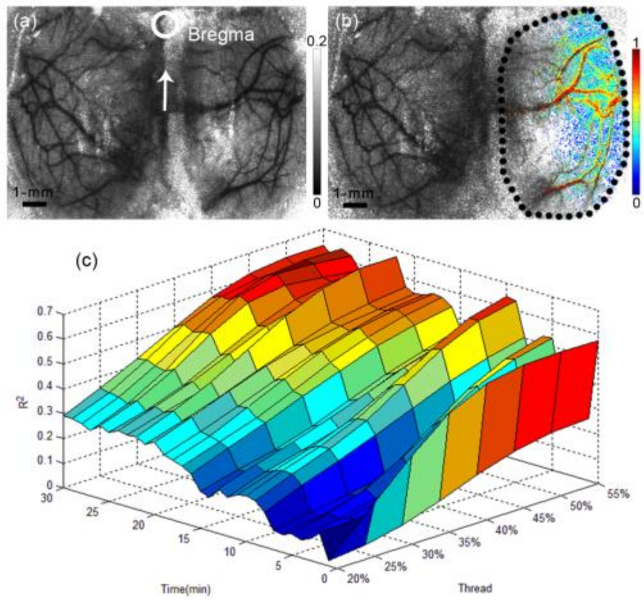

Figure 2 (a) Laser speckle contrast image at the baseline of the surgery, (b) Overlapped image with CBF reduction area plotted in pseudocolor, (c) Variation of the correlation coefficient R^2 after the occlusion in 30 minutes in different threads.

this study, R^2 reached a maxima at $t = 22$ min for $\varepsilon = 55\%$ ($R^2 = 0.677$, $P < 0.05$). Noted that R^2 reached the maximum when $\varepsilon = 50\%$ ($R^2 = 0.580, P < 0.05$) at $t = 0$ min, which was in line with the previously published paper [11].

B. *Prognostic of cerebral blood flow in different vessels*

We further investigated the different prognostic of the CBF in different vessels. Fig. 1 shows CBF images in pseudo-color at different time points of the surgery. As it displays, there is no remarkable difference of CBF in bilateral hemisphere when CCA was ligated. While the ICA was occluded, the right CBF immediately dropped significantly, resulting in the ischemic stroke.

Three regions of interests (ROIs) were selected for quantitative CBF analysis, including the vein in right hemisphere (RV), the artery in right hemisphere (RA) and the capillaries in the core area of the right hemisphere (RC) (see Fig. 3). The relative CBF velocities in each ROI were calculated respectively with the contrast values normalized by the baseline level. Fig. 4(a) shows the relative CBF in different vessels during the first 30 minutes immediately after the occlusion ($n = 14$). Right after the occlusion of ICA, the CBF in the vein, the artery and the capillaries of the right hemisphere decreased to 56.8 ± 7.9% ($P < 0.05$), 46.5 ± 8.5% ($P < 0.05$) and 52.3 ± 7.5% ($P < 0.05$), respectively. At the beginning of MCAO, the CBF fluctuated remarkably in all vessels after an initial drop. Then CBF velocities gradually recovered. Different dynamics of CBF change could be observed in different vessels (Fig. 4(a)), and the CBF in ipsilesional vein showed evident recovery, while the CBF in capillaries in the right hemisphere was more stable in the early stage of the MCAO. The CBF recovered to 64.4 ± 9.2% ($P < 0.05$), 48.6 ± 7.9% ($P < 0.05$) and 55.0 ± 6.6% ($P < 0.05$) in the ipsilesional vein, the artery and the capillaries, respectively at 30th min after the MCAO.

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

Further, we analyzed the correlation coefficient R^2 between the normalized blood flow velocity and infarct volume in each ROI defined in Fig. 3. Fig. 4(b) shows the minute-by-minute correlation coefficient R^2 within the first 30 minutes after the occlusion in the selected artery, vein and capillaries. Though the R^2 values between CBF and the final infract volume were all remarkably significant (artery: $R^2 = 0.606$, $P < 0.05$; vein: $R^2 = 0.632$, $P < 0.05$; capillaries: $R^2 = 0.656$, $P < 0.05$) immediately after the occlusion, which is consistent with our previous report [18], their correlations within the first 10 minutes showed high variance. Such a prognostic was more stable after 15 minutes of MCAO. It is noted that artery R^2 reached the maximum at the 6th minute after occlusion ($R^2 = 0.795, P < 0.05$) and the vein R^2 reached the maximum at the 22nd minute after occlusion ($R^2 = 0.724, P < 0.05$), while the capillary R^2 reached the maximum at the 10th minute after occlusion ($R^2 = 0.718, P < 0.05$). These results indicated that the artery blood flow was more prognostic in rodent MCAO injury.

Discussions and Conclusions

In this study, we recorded the real-time full-field CBF images during the MCAO rat model with a miniaturized head-mounted LSI system. The data were recorded continuously from the baseline up to 30 minutes after the occlusion of ICA in surgery. And the infraction volume was assessed 24 hours after the surgery. The correlations between CBF changes in different ipsilesional vessels and final infract volumes were analyzed, which is aiming to find out the more prognostic marker for predicting the ischemic lesion.

By analyzing the correlation between the infarct volume 24 hours after stoke and the size of area with different CBF drop (i.e. 20~55%), we found that 45~55% CBF drop is more effective in predicting the severity of brain injury. And the overall R^2 showed more fluctuation at the beginning of MCAO (i.e. 0~15 min) before it became stable (15~30 min).

We further compared the prognostics of different blood vessels. Three ROIs representing the ipsilesional artery, vein and capillaries were selected for analysis. The correlation

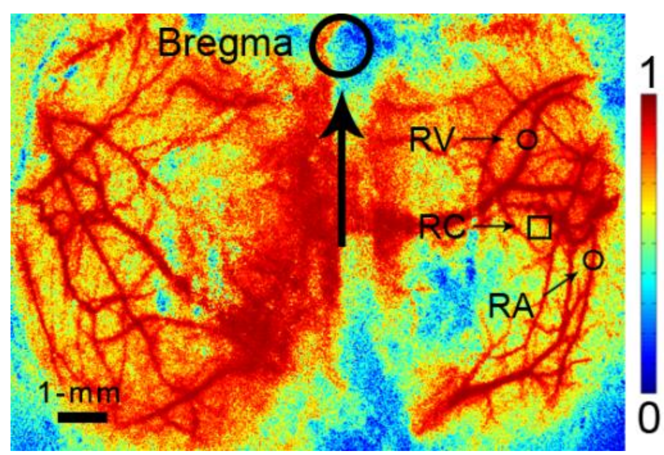

Figure 3 Three ROIs are identified in its pseudo-color image: RV standing for region of vein in right hemisphere, RA standing for region of artery in right hemisphere and RC standing for region of capillary in right hemisphere.

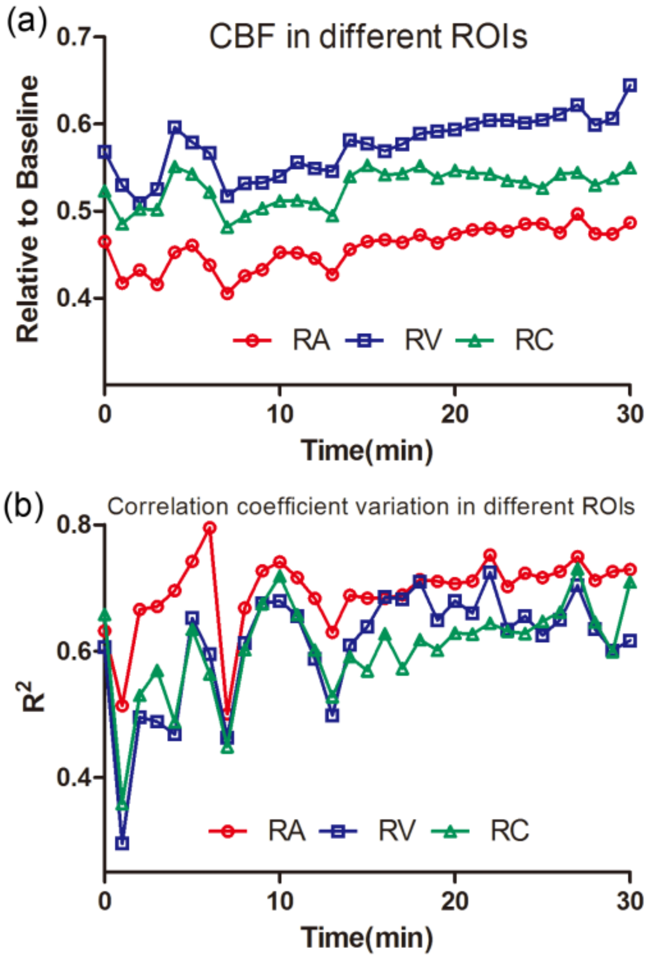

Figure 4 (a) CBF in different ROIs during 30 minutes immediately after the occlusion (n=14), (b) Variations of correlation coefficients in different ROIs between infarct volume and relative CBF velocities during 30 minutes immediately after occlusion.

between the average CBF level in each ROI and the lesion volume were assessed minute by minute after the stroke. Though the CBF dropped obviously in all ipsilesional blood vessels, then it gradually recovered. However, ipsilesional artery CBF consistently showed higher R^2, indicating the more prognostic value, which might be due to the direct occlusion of the ICA in the model.

This preliminary study further proved the prognostic value of early CBF change in ischemic stroke, and the effectiveness LSCI as an intraoperative blood flow monitoring, which is supporting our previous conclusions. Furthermore, this study showed different prognostic value of the CBF change in different cerebral vessels. Besides, both CBF reduction area and average CBF velocity are demonstrated to be correlated with the infraction volume. The results implied that the artery CBF change could be more prognostic than the veins and capillary bed, and the time window with more stable CBF 15 minutes after the MCAO is recommended for monitoring.

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