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Spatiotemporal Changes of Cerebral Blood Flow following Hemorrhagic Stroke by Laser Speckle Imaging

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Abstract—**Hemorrhagic stroke accounts for more than 15% of all stroke hospitalization with much higher mortality than ischemic stroke. In this study, we investigated the spatiotemporal changes of the cerebral blood flow (CBF) following intracerebral hemorrhagic (ICH) stroke by laser speckle imaging. Adult male C57BL/6 mice were divided into ICH group (n=7) and saline group (n=4). CBF images were recorded before injury, 30 minutes, 24, 48, and 72 hours after stroke. Results showed that both ipsilateral CBF and contralateral CBF significantly reduced in two groups, which suggested that the mass effect was the dominant factor in the early stage of hemorrhagic brain injury. In ICH group, although the lesion was mainly around the injection location at first, hematoma could last for a long period of time and cause a secondary brain injury. The preliminary results showed that laser speckle imaging demonstrated its reliability with high spatiotemporal resolution in imaging mouse CBF with hemorrhagic stroke.**

I. Introduction

INTRACEREBRAL hemorrhage (ICH) is a devastating clinical condition, accounting for more than 15% in all stroke patients [1][2]. Although hemorrhagic strokes are less common than ischemic strokes, they usually cause severer neurological disturbances than other types of strokes such as ischemia [3]. ICH is also responsible for severe damage to brain tissue that can leave individuals disabled or with difficulty in speaking, swallowing, thinking properly, or daily activities.

Monitoring cerebral blood flow (CBF) change is very important in the study of brain vascular diseases including ICH. Also, knowing the impact of ICH on the cortical vascular system would help us find out when and what treatment should be administrated after ICH occurs. Though ICH has a much higher death rate than ischemic stroke, researches on ICH are far less than ischemic stroke because there was no advanced technology to monitor CBF changes after hemorrhagic stroke. In recent years, a two-dimensional blood flow imaging technique, i.e. laser speckle imaging (LSI), has gained success in the study of CBF changes. Compared with traditional laser Doppler flowmetry (LDF), LSI has its unique advantages[4][5], which could provide two dimensional global CBF velocity information in real time. With the help of LSI technology, researchers are able to study the details of the spatiotemporal changes of CBF, for example, the study of ischemic stroke based on rodent model [6].

So far, most studies on ICH focused on mechanism of brain injury and neurological function improvement after stroke. The dynamic changes of CBF after ICH have not been well uncovered. In this study, we implemented LSI to quantify the changes in CBF at different cortical regions following the stroke up to three days in a mouse model. And we further investigated i) what were the spatiotemporal patterns of CBF following ICH? and ii) how were ICH and saline treated mice different in the aspect of CBF?

To answer these questions, we adopted a mouse model of ICH[1] by injecting autologous blood into the right striatum of mouse brain, which could mimic hemorrhagic stroke. In the control group, mice were injected with saline in the same location. During the experiment, we utilized temporal laser speckle image contrast analysis (tLASCA) [7] to find the long-term impact of hemorrhagic injury on the CBF velocity changes at different cortical regions before and after the injury up to three days.

II. Materials And Methods

A. Experimental Design

Studies were carried out on adult male C57BL/6 mice weighing 18 to 24 grams (Slac Laboratory, Animal, Shanghai, China). The experimental protocols were approved by the animal care and usage committee of Med-X Research Institute, Shanghai Jiao Tong University. The mice were divided into ICH group (n=7) and saline group (n=4) with injection of autologous blood and saline, respectively. CBF images were recorded at five time points, i.e. baseline (before surgery), 30 minutes, 24, 48, and 72 hours after surgery.

During the surgery and CBF imaging, each mouse was anesthetized with sodium pentobarbital (60 mg/kg). Before surgery, a midline incision was made over the scalp and the tissues were cleaned to expose the surface of the skull with a blade. The imaging field of the mouse skull was approximately 10 mm×8 mm. The skull of mouse was kept intact for long-term observation. It was covered with glycerol before CBF imaging to reduce the specular reflections. After obtaining the speckle images of baseline, we injected the autologous blood or saline into the mouse brain to conduct the

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surgery. Detailed procedures of the mouse model will be discussed in the following subsection. Then we recorded speckle images 30 min after surgery. After that, the scalp was closed with suture and cleaned with ethyl alcohol, and then the mouse was removed from the stereotaxic frame and placed into a humidity and temperature controlled incubator. After 24 h, the mouse was anesthetized again for CBF imaging as the first day data. Such imaging procedures were repeated at 48 h and 72 h.

During the surgery and imaging, mouse was constrained in a stereotaxic frame (Steolting Co. the 51603 Dual Manipulator Lab Standards™, U.S.A). A heating pad and DC temperature control module (FHC Inc., Bowdoinham, U.S.A) were used to maintain the temperature of mouse at 37.0±0.2°C throughout the experiment. Mice were allowed free access to water and food during the awake states.

B. Mouse Model of ICH

Mouse model of ICH was adopted from Rynkowski *et al.* [1], i.e. double-injection model. The hemorrhagic stroke was created by injecting the autologous blood into mouse striatum in two stages, which could closely mimic the natural course of human ICH.

A 30-gauge stainless steel cannula was introduced through a burr hole into the right striatum of the mouse under anesthesia, 1 mm posterior of bregma, 2 mm lateral to the midline, and 2.5 mm into the brain. In the first stage, 15 μl of blood were injected at a rate of 3 $\mu l / \min$ into the brain. The rest 15 μl blood were injected 2 minutes later with the same rate as in stage 1. The cannula was slowly removed 10 minutes after stage 2.

C. Imaging Procedures

The laser speckle images were acquired at 23 fps (exposure time T=5 ms) with a 12 bit CCD camera (Pixelfly QE, Cooke, U.S.A) mounted on a trinocular stereo microscope (XYH-05, Shanghai Optical Instrument Factory, China) over the skull illuminated by a semiconductor laser diode (780±2 nm, Shanghai Forward Optoelectronics Co., Ltd., China). Resolution of the imaging field was 696 pixels×512 pixels. For each trial we recorded 200 consecutive frames of laser speckle images. The stereotaxic frame, the microscope, and the diode laser source were all fixed on an optical table during the entire experiment to ensure a stable optical imaging condition.

D. Data Analysis

1) Theory of temporal laser speckle contrast analysis (tLASCA): When a highly coherent laser light illuminates a rough surface, the interference of the reflected or scattered light results in granular pattern known as laser speckles [8][9]. In a similar way, when laser irradiates the material with moving particles, such as blood cells, we could also detect the speckles. The speckle pattern contains the motion information of scattering particles. The velocity of scattering particles affects both intensity and frequency of speckles which could be extracted by analyzing the temporal or spatial statistics of the speckles. Briers *et al.* [5] introduced the laser speckle contrast analysis (LASCA) algorithm which related speckle pattern to the velocity of the scattering particles. LASCA was defined as the ratio of the standard deviation to the mean of the intensity in the image [10]:

$$0 \le K_s = \frac{\sigma_s}{\langle I \rangle} \le 1 \tag{1}$$

Speckle contrast K_s was linked to the velocity by:

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

where T is the exposure time of the CCD, and the autocorrelation time τ_c is inversely and linearly proportional to the mean velocity of the blood flow [5].

To avoid the loss of spatial resolution due to the averaging over the sliding window in LASCA, Cheng *et al.* [7] developed temporal laser speckle contrast analysis (tLASCA) algorithm, which was based on the first-order temporal statistics of time-integrated speckle patterns. tLASCA was defined as:

$$K_t^2(i,j) = \frac{\sigma_t^2(i,j)}{\langle I_t(i,j) \rangle^2} = \frac{\langle I_t^2(i,j) \rangle - \langle I_t(i,j) \rangle^2}{\langle I_t(i,j) \rangle^2} \tag{3}$$

where $I_t(i,j)$ is the intensity of each pixel in a raw speckle image sequence and K_t^2 is inversely proportional to the velocity of the scattering particles. We could use $1/K_t^2$ to calculate the relative changes of CBF in our experiment. Comparing with (2), tLASCA has a higher spatial resolution which is able to provide more details in small vessels [7].

2) Image Processing: The speckle images for tLASCA were 200 frames in each trial. We defined the relative CBF change as the ratio of $1/K_t^2$ to the corresponding mean value of baseline. Considering the variability in the spatial distribution of cortical blood vessels of different mice, we selected the average CBF in several cortical regions instead of certain vessels to analyze CBF changes. Six regions were selected symmetrically from both hemispheres of each mouse, i.e. Right – Top, Right – Middle, Right – Bottom, Left – Top, Left – Middle, and Left – Bottom as illustrated in Fig.1, and the injection location was designated in the Right – Middle region. Considering the individual difference in mouse cortex, the selected six regions were slightly adjusted to include the major blood vessels. Average CBF of the three regions in each hemisphere represented ipsilateral CBF (right) or contralateral CBF (left).

E. Statistical Analysis

Significance levels of the differences in CBF changes in six regions at each time point were tested by one-way analysis of variances (ANOVA). In each group, tLASCA values were analyzed with student *t*-test (ipsilateral vs. contralateral) for six cortical regions. At each time point, tLASCA values were

analyzed with *t*-test (ICH vs. saline) for six cortical regions. Average tLASCA values between two experiment groups were also tested for ipsilateral CBF and contralateral CBF at each point of time. Values of $P<0.05$ were regarded as significant differences.

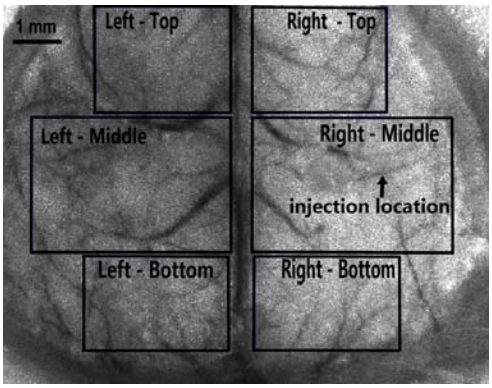

Fig. 1. Illustration of the cortical regions to be analyzed in CBF change.

III. Results

By analyzing the tLASCA values before and after ICH occurred, we found distinct CBF changes due to the injury. Fig.2 illustrated the CBF changes up to 3 days of two mice from ICH group and saline group, respectively.

Changes of ipsilateral CBF and contralateral CBF in both ICH and saline groups are shown in Fig.3. In saline group, the experimental protocols were slightly changed to lower the probability of inflammation occurrence and we did not collect the 48 h data of 3 mice in saline group. Therefore, average CBF at 48 h in saline group was not included in Fig.3. By comparing CBF in two hemispheres in two groups, we had the following findings:

30 min after surgery, ipsilateral CBF in ICH group reduced to 30-50% of baseline, which was much more than the decrease in the contralateral hemisphere. Within the days after stroke, ipsilateral CBF in ICH group firstly increased at 24 h, then decreased at 48 h and further rebounded at 72 h.

At 72 h, ipsilateral CBF in saline group exceeded the baseline level, while contralateral CBF was close to the baseline. Whereas CBF of the ICH mice in every selected region did not return to the baseline level.

By statistical analysis of average tLASCA values on both hemispheres at each time point in both groups, we found that 30 min after surgery, the average CBF velocities of ICH group significantly differed from that of saline group in the left hemisphere. At 72 h, the average CBF velocities of ICH group in both hemispheres significantly differed from saline group.

IV. Discussions And Conclusions

Our experiment showed that CBF in both hemispheres significantly reduced in either ICH or saline group, which suggested that the mass effect was the dominant factor in the early stage of hemorrhagic brain injury. However, ipsilateral CBF of the ICH mice reduced significantly more than contralateral CBF after stroke, which demonstrated the impact of hematoma on ipsilateral CBF was severer than contralateral CBF at first. Nevertheless, the impact of saline and the following edema on ipsilateral CBF and contralateral CBF was comparable.

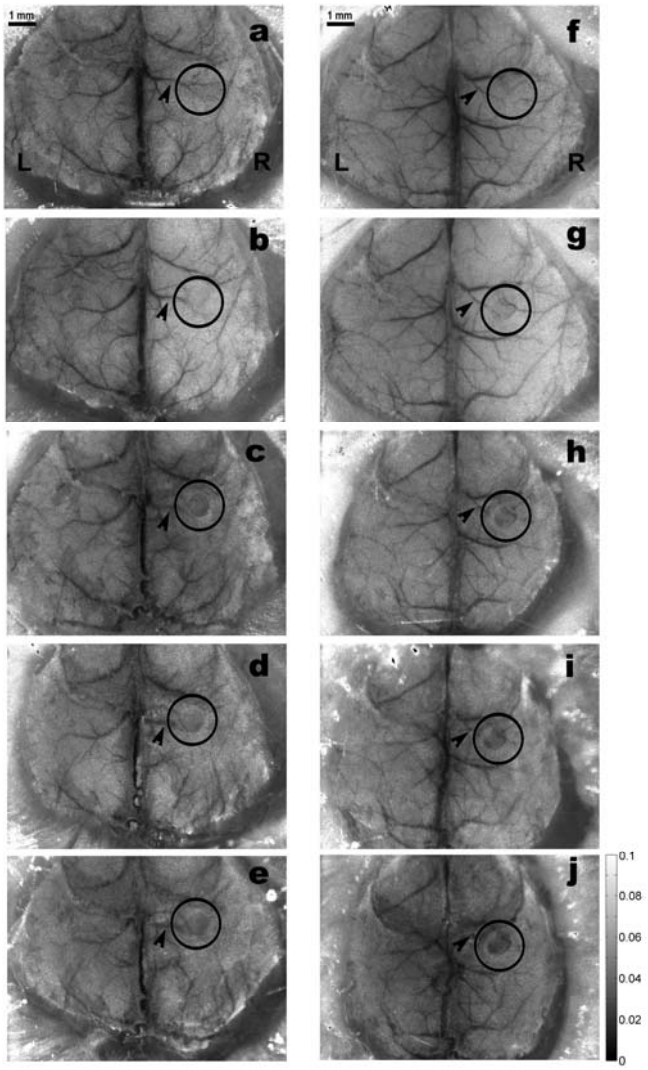

Fig. 2. Laser speckle images of one ICH (a, b, c, d, e) mouse and one saline (f, g, h, i, j) mouse. Circles designate the injection locations of blood or saline. Arrows point to the most adjacent blood vessels to the hemorrhagic locations. R refers to right (ipsilateral hemisphere), L refers to left (contralateral hemisphere). a, f – baseline, b, g – 30 min, c, h – 24 h, d, i – 48 h, e, j – 72 h.

Contralateral CBF of saline group declined more quickly than that of ICH group after surgery. But the recovery of CBF in both hemispheres in the saline mice was much better than the ICH ones. We speculated that the acute influence of edema in saline group should be severe in contralateral hemisphere, and the lesion extended through forebrain and caused serious edema on both hemispheres in the first few days. Meanwhile, hematoma in ICH group was mainly

around the injection location at 30 min, which did not cause an obvious impact on contralateral CBF in a short term. Nevertheless, hematoma lasted for a long period of time with its impact gradually spread to both hemispheres after 24 h. Without a secondary lesion, the saline mice showed a clear trend of CBF recovery in the first three days. Comparatively, intracranial hematoma could release cytokines and chemokines [11], and cause secondary lesion. This injury might be the main reason for the poor recovery of CBF in the ICH mice.

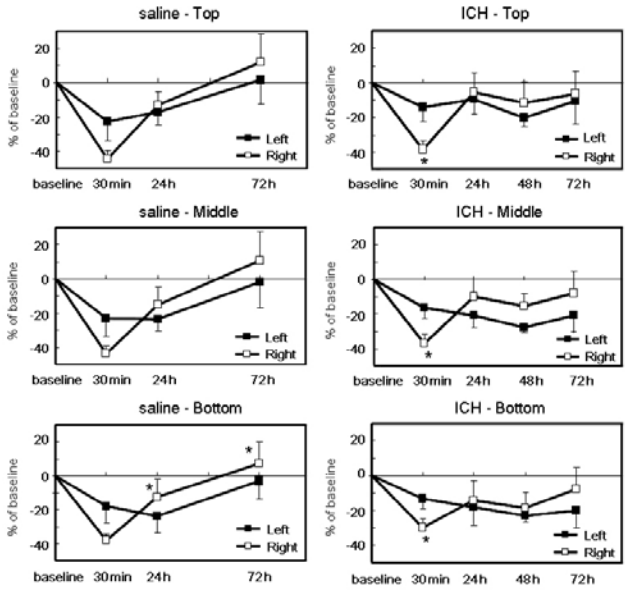

Fig. 3. CBF changes in ICH group and saline group. Values were as percentage decrements (expressed as mean±SEM) compared with the average level in baseline. In ICH group, CBF data at baseline, 30 min and 24 h were from all seven mice. While at 48 h and 72 h, we only recorded CBF from five mice. In saline group, CBF data at baseline, 30 min, 24 h, and 72 h were from all four mice. While at 48 h, we only recorded CBF from one mice (not shown in the figure). * shows the significant hemispheric difference of CBF change ($P<0.05$).

In addition, some mice showed evident morphological changes of the blood vessels after hemorrhagic stroke, which was due to a collateral circulation open. Both hematoma and edema could cause hypoperfusion around the hematoma, which would increase the intracerebral pressure and then cause dilation compensatory phenomenon in blood vessels. Not surprisingly, vascular morphological changes were more commonly observed in ICH group than in saline group.

Although mouse with intact skull could survive longer in our experiment, inflammation on mouse skull was still the biggest challenge for a longer period observation, which would prevent penetration of laser and degrade the imaging quality more than 3 days after surgery. Further study with cranial window over the skull will extend the image acquisition up to seven or more days so that we can get more details of CBF velocity changes after hemorrhagic stroke.

In conclusion, we successfully applied laser speckle imaging technology to achieve long-term monitoring of cerebral blood flow changes in mice with ICH, which is important in the study of hemorrhagic stroke. We observed that velocity of ipsilateral CBF significantly reduced after surgery in both the ICH mice and the saline mice. For the ICH mice, injury lasted longer along with a poor recovery of CBF in 3 days since the hemorrhagic stroke occurred; For the saline mice, the impact of edema on CBF lasted for less than 3 days while CBF gradually returned to baseline level.

LSI provided a powerful tool to quantify and visualize the cortical blood flow and acquired the entire cortical vasculature morphology information simultaneously. Laser speckle imaging of mouse CBF was reliable with high spatiotemporal resolution, which was able to accurately reflect the abnormal cerebral hemodynamic changes of areas around the hemorrhagic location, and also the vascular morphological changes. However, the physiological mechanisms of CBF changes after hemorrhagic stroke need to be further explored.

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