Real-time monitoring of cerebral blood flow by laser speckle contrast imaging after cardiac arrest in rat

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***Abstract*— Cardiac arrest (CA) results in global brain ischemia. To explore the role of cerebral blood flow (CBF) during ischemia, laser speckle contrast imaging (LSCI), a full-field high-resolution optical imaging technique, was used for real-time monitoring of the fluctuations of CBF in a rat model of asphyxial-CA. The temporal changes of CBF were characterized and the relationship between CBF and mean arterial pressure (MAP) was evaluated. Asphyxial-CA led to transient CBF dysregulation, manifested by changes in CBF velocity were significantly impacted by MAP. Hyperemia is aligned with a bolus injection of vecuronium, the first two minutes of asphyxia, the time of epinephrine injection and cardiopulmonary resuscitation, and then lasted for 13 min after the return of spontaneous respiratory (ROSC), followed by hypoperfusion about 55-70% of baseline level no later than 40 min after ROSC. Interestingly, we found that the velocity of venule blood flow increased more than that of the arteriole blood flow during the hyperemia (176% vs 120%). Our study, for the first time, shows real-time CBF changes during and immediately after asphyxial-CA, with high spatial and temporal resolution images. The quantified cerebro-vascular response during the different phases of recovery after CA may underlie the mechanism of injury and recovery after brain ischemia. The study provides a new technique to study the neurovascular coupling and metabolic regulation of CBF after CA.**

I. INTRODUCTION

Cardiac arrest was a dominant cause of approximately 324,200 deaths and disabilities in the United States in 2015 and the survival rate from CA is only 10.6 % [1]. Since CA leads to a global cerebral hypoxic-ischemic injury, understanding the mechanisms of the functional disruptions caused by CA is essential for the development of improved diagnostic and therapeutic solutions. Cerebral blood flow (CBF) provides an energy supply to the brain and therefore plays a critical role in the global ischemia caused by CA. Characterization of the effect of CBF can provide a better understanding of the mechanism of ischemia. CBF after CA is distinguished by a short transient hyperperfusion followed by a sustained hypoperfusion [2, 3]. However, due to undeveloped techniques, the real-time changes of CBF after CA, especially before the return of spontaneous circulation (ROSC), have not yet been reported.

Although laser Doppler flowmetry (LDF) has been one of the most commonly used techniques to monitor CBF during laboratory and pre-clinical studies in the past two decades, the information obtained is spatially constrained. Conversely, a less expensive optical imaging methodology, laser speckle contrast imaging (LSCI) uses optics to obtain a two dimensional, wide field view of the cortex to monitor the spatio-temporal progression of CBF [4]. The temporal and spatial precision of LSCI exceeds that of LDF [5, 6]. The equipment required for LSCI is minimal and the setup is simple. Furthermore, injection of a contrast agent is not required in LSCI. LSCI not only displays images, but also quantifies blood flow velocity, blood volume, vessel dilatation/constriction responses and deoxy-hemoglobin saturation changes. These advantages make LSCI suitable for monitoring the CBF information in the clinically relevant rat model of asphyxial-CA.

In this study, we utilized a self-developed LSCI system [7-9] to obtain detailed real-time CBF information during and after asphyxial-CA in a rat model. The alterations of CBF in cortical arteries, cortical veins, and capillaries in the primary motor cortex were quantified.

II. METHODS

A. Animal Preparation

All experiments were performed using a protocol approved by the University of Maryland Animal Care and Use Committee. Three adult Wistar rats (4*50 - 500 g*) were used. One day before cardiac arrest, the rats were anesthetized by 1.5% isoflurane and fixed in a stereotactic frame (David Kopf instruments, Tujunga, California, USA). A midline incision was made over the scalp. A *5 mm × 5 mm* area centered at AP, -2.5; ML, -2.5 was thinned using a high speed dental drill (Fine Science Tools Inc. North Vancouver, Canada), until the cortical vessels were clearly visible. Bone wax was applied to the thinned skull to keep the area of interest moist. The cranial window was encircled by a cylinder base (laboratory-designed, height: 4.2 mm, radius: 5.5 mm,

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thickness: 0.5 mm) that was connected to the imaging system. The cylinder base was fixed on the skull by dental cement. All procedures were performed under standard sterile conditions. Rectal temperature was maintained at *36.5-37.5˚C* using a heating pad for the duration of the surgery.

B. Asphxial-CA Animal Model

We used the previously developed experimental protocol to induce cardiac arrest and resuscitation in rats [10-14]. On the day of CA, 1.5% isoflurane mixed with 1:1/oxygen:nitrogen was delivered by a ventilator to initiate anesthetization after tracheal intubation. To administer drugs and monitor mean arterial pressure (MAP), cannulations of the femoral artery and vein were performed before the initiation of CA. After that, a 5-min baseline of LSCI images with 1.5% isoflurane was recorded. The washout period with 100% oxygen for 2 mins and room air for 3 mins followed the baseline recording in order to prevent the effect of anesthesia on the images. During the last 3 mins of the washout period, the rats were paralyzed via an i.v. bolus injection of vecuronium (2 mg/kg) followed by clamping the breathing circuit and disconnecting the ventilator for 9 mins to induce cardiac arrest. We recorded the time of cardiac arrest when pulse pressure <10 mm Hg. Cardiopulmonary resuscitation (CPR) was initiated immediately after asphyxia with oxygenation, sternal chest compression, epinephrine and NaHCO3 until MAP > 60 mmHg, which we defined as return of spontaneous circulation (ROSC). The arterial blood gases were measured from the femoral artery via cannulation at 20 mins after ROSC.

C. Laser Speckle Contrast Imaging and Image Processing

The miniature laser speckle imaging system was used to record the CBF. It was connected to an 8-bit COMS camera to capture the image, which was mounted on the cylinder base over the skull, and a laser diode (780 nm; 10 mW; L780P010, Thorlabs) to illuminate the region of interest (ROI), which was powered by a driver module (LDC220C, Thorlabs). Laser speckle images (640×640 pixels) were acquired at 50 fps (exposure time T=5 ms). In each trial, we recorded 320 consecutive frames of speckle images. Images were acquired 10 min prior to cardiac arrest (baseline), for 30-min continuously after the isoflurane washout, followed by 15-min intervals on and off until 4 hours after ROSC, then 2-4 recordings from 4h to 36h after ROSC. After registration, laser speckle contrast analysis was implemented to obtain the CBF information. The relative CBF (rCBF) velocity changes in the selected ROI was acquired throughout the experiment. The rCBF changes in cortical arteries and cortical veins were calculated and averaged. After selecting a ROI in the center of the primary motor cortex (M1) with a size of 1 mm × 1 mm (centered at AP, -1.5; ML, -0.5), we further acquired the mean rCBF velocity changes of capillaries in M1 after eliminating cortical vessels from the images.

III. RESULTS

A. Real-time imaging of CBF in asphxial-CA model

Fig.1 shows representative LSCI images at the time points of interest. There were clear changes in CBF during asphyxia. Rapid decrease of CBF occurred immediately after clamping the ventilation tubes (Fig 1. III-V vs I). The pulse pressure decreased below 10mmHg after 3min 40sec of asphyxia until resuscitation at 10 min, during which time the CBF remained stagnate. Immediately following CPR, CBF returned to the baseline level within 1 min (VI), then increased far above baseline (VII-XI). CBF decreased below baseline no later than 40min after ROSC (X-XI), then returned back to baseline at 30 hours after CA.

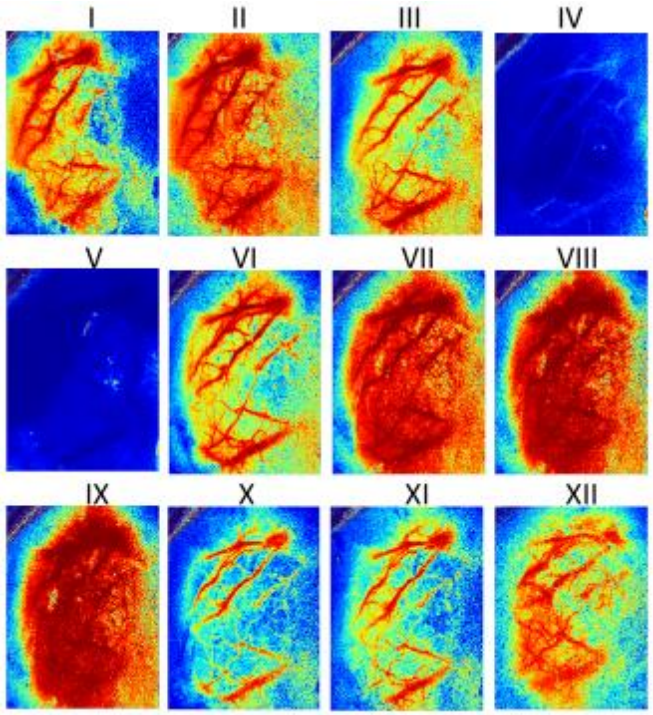

Fig. 1. CBF representative images at typical time points of events. I: baseline; II: 3 min before asphyxia (washout period); III-V: 1, 4, 7 min after asphyxia; VI-IX: 1, 4, 7, 11 min after ROSC; X-XII; 40 min, 120 min and 30 h after ROSC.

B. Characterization of CBF fluctuation in the asphxial-CA model

CBF is relatively independent of changes in MAP within a certain range. The lower and upper limits of CBF autoregulation correspond to a MAP of approximately 50–60 and 150–160 mmHg, respectively [15]. Outside of this range, CBF becomes pressure passive as the arterioles cannot contract or dilate further to maintain consistent flow, which is called dysregulation.

During CA, the CBF was minimized or completely disrupted. A delayed hypoperfusion occurred 15-45 min after ROSC and continued for several hours thereafter (Figs.1-2).

Fig. 2 shows the correlation of CBF and MAP in the first hour of recording from a representative rat. There were four hyperemia peaks of CBF corresponding to four surcharges of MAP, indicating the dysregulation of CBF.

The 1st peak is aligned with the bolus i.v. injection of vecuronium, which led to an abrupt increase in MAP. The CBF of the artery, vein and M1 region increased to the same amplitude (about 125% of the baseline). The 2nd hyperemia peak occurred at the time of asphyxia. CBF was dysregulated

even though MAP had fallen into the range of autoregulation (under 120 mmHg in this case). The 3rd hyperemia peak was relatively brief and occurred immediately after ROSC. It lasted for one minute. The 4th hyperemia peak occurred simultaneously with the sustained increase of MAP. This peak in our experiments was consistent with the results previously reported by Kagstrom et al [3], which lasted for 15-30 min after ROSC characterized by CBF 2-3 times higher than baseline.

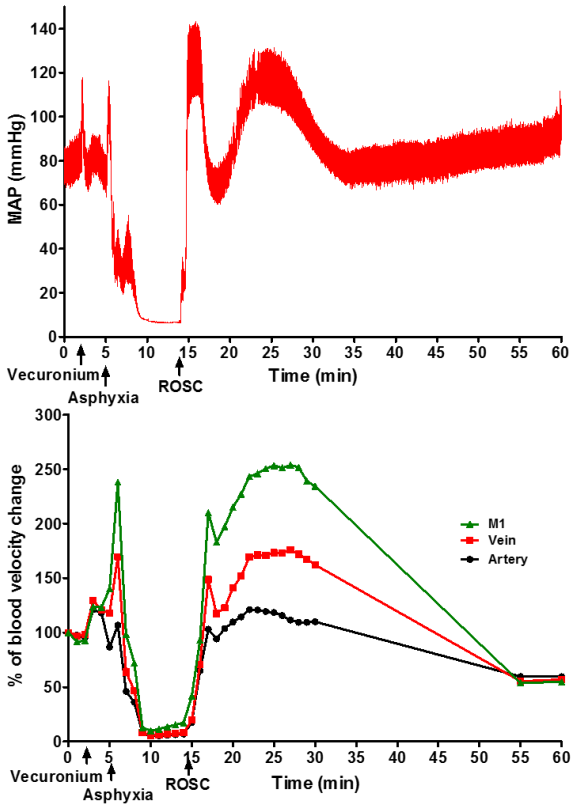

Fig. 2. Passive changes of CBF to MAP from one representative rat. MAP was recorded continuously for more than one hour. CBF was recorded continuously for 30min starting from isoflurane washout and periodically thereafter. There were four MAP surcharges. The first two occurred before CA, corresponding to i.v. bolus injection of vecuronium and asphyxia. The 3rd one was presented after epinephrine injection and CPR. These three peaks were very brief. The fourth peak lasted for more than 5min after ROSC. CBF is shown in the lower panel, four peaks occurred simultaneously with the four surcharges of MAP.

During the 2nd, 3rd and 4th hyperemia periods, CBF dysregulation was characterized by a distinctive increase of flow velocity between the artery and vein. It was interesting to find that CBF in the capillaries of the M1 region and the veins increased more than that of the arteries.

C. Charaterization of vessel diameter changes in the asphyxial-CA model

The change in vessel diameter of the middle cerebral artery (MCA) and three primary veins from one representative rat are shown in Fig.3. Both the artery and veins contracted greatly after 1-2 minutes of asphyxia. The vessel diameters were reduced by more than 50% of their original size. At the time of CA (3min 40s after asphyxia), the diameter of the vessels returned to baseline.

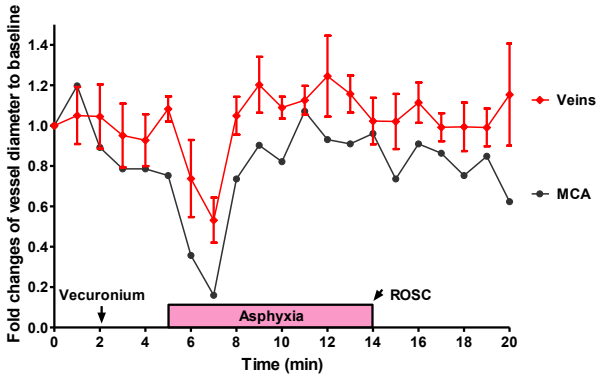

Fig.3. Representative changes of vessel diameter during asphyxia CA.

IV. Discussion

In this study, we used the new technique of LSCI to examine the major fluctuations of CBF in real-time, before, during and after CA in a rat model of asphyxial-CA. To our knowledge, this is the first report of real-time CBF changes during asphyxial-CA with high spatial and temporal resolution images (Fig. 1).

The ability to non-invasively monitor and quantify detailed and longitudinal hemodynamic responses vessel by vessel in the same animal over time is important for a better understanding of the underlying mechanism, improved diagnostics, and optimization of future treatment and management of brain ischemia. The high temporal resolution of LSCI identified, for the first time, that the CBF dysregulation time point occurred as early as the initiation of asphyxia. As seen in Fig.2, the first three instances of hyperemia dysregulation of CBF are so brief and abrupt that only techniques such as LSCI could clearly capture such a transient change. The quantified cerebro-vascular response during the different phases of recovery after CA may underlie the mechanism of injury and recovery after brain ischemia.

There are multiple mechanisms for the dysregulation of CBF in the asphyxial-CA model. For the 1st hyperemia dysregulation, CBF changes were not compensated by autoregulation upon rapid variations in arterial pressure since the vessels require time to adjust to the arterial pressure changes [16, 17]. Of course, the increase of CBF at this time point may also be due to contributions of the anesthesia washout or FiO2 changes. In asphyxial-CA, before CA is achieved, events such as systemic hypoxemia, hypercapnea, acidosis, hypotension and bradycardia occur [18]. These factors may have caused the 2nd hyperemia dysregulation. We believe the 3rd hyperemia peak was the response of CBF to epinephrine and CPR [19]. The 4th hyperemia peak has been well studied, with most knowledge inferred from a stroke model. Human stroke models demonstrate that the duration of post-ischemic hyperperfusion is proportional to the time that ischemia is maintained [20]. Additionally, hyperemia may be associated with irregular vasodilation in the region of ischemic insult rather than increased usage of oxygen or glucose. Irregular vasodilation may be attributed to ischemia induced lactic acidosis or vasoactive mediators, which might lead to additional delayed injury or bad outcome [21, 22].

The mechanisms leading to post-ischemic hypoperfusion are still unclear. A study in cats showed that both extracranial and intracranial arteries are involved in the increase in cerebrovascular resistance, which may be due to microvascular compression and vasospasm, that follows ischemia-reperfusion [23]. However, post-ischemic hypoperfusion may also be related to a decrease in metabolic demand and synthesis of parenchymal and endothelial vasoactive agents [24]. The mechanism underlying the regulation of vessel constriction and dilation needs to be further investigated in the future.

The wide field and high spatial resolution of LSCI enable us to distinguish regions and subtleties of CBF responses to brain ischemia. We found the changes in amplitude of CBF from arteries and veins down to the capillary level were distinctive in the 2nd, 3rd and 4th hyperemia stages: the CBF in veins and capillaries of M1 region increased more than that of arteries, which would lead to the drainage of blood directly out of the brain and may be one of the mechanisms of ischemia.

V. Conclusion

In summary, by taking advantages of the high temporal and spatial resolution of LSCI, we identified the CBF dysregulation time point to be as early as the initiation of asphyxia, and the changes in amplitude of CBF in veins and arteries were distinctive in the hyperemia stage, with a larger increase in CBF in veins and capillaries of the M1 region than in arteries. These novel findings will enrich our knowledge about the injury and recovery after brain ischemia.

Besides the aforementioned applications, the other parameters of CBF such as blood volume, vessel dilatation/constriction responses and deoxy-hemoglobin saturation changes can also be measured simultaneously by the LSCI imaging platform. This would open a new avenue to study the neurovascular coupling and metabolic regulation of CBF in the context of cardiac arrest, which can be useful in improving our understanding of vascular responses under pathologic and physiological conditions and ultimately facilitating clinical diagnosis, monitoring and therapeutic interventions of neurovascular diseases.

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