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Cerebral Blood Flow Changes during Rat Cardiopulmonary Bypass and Deep Hypothermic Circulatory Arrest Model: A Preliminary Study

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Abstract—**Cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) are important techniques often used in complex cardiac surgery for neonates and infants heart diseases. Cerebral blood flow (CBF) serves as an important physiological parameter and provides valuable hemodynamic information during the surgery. Laser speckle imaging (LSI), as an optical imaging technique, can provide full-field CBF information with a high spatiotemporal resolution. In this preliminary study, we acquired the real-time CBF images with a self-developed miniaturized head-mounted LSI system during the whole CPB/DHCA rat model. Relative CBF velocity in veins and arteries in bilateral hemispheres dropped significantly during cooling period and reached to nearly zero during arrest period ($n = 5$). More interestingly, two rats showing more dramatic CBF variations in veins than in arteries during cooling period exhibited severe cerebral edema after surgery. The real-time full-field CBF imaging during the CPB/DHCA surgery could add more insights into the operation and be utilized to study the surgical protocols with the ultimate goal of reducing neurologic injury after surgery.**

I. Introduction

Cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) are important techniques often used in complex cardiac surgery for neonates and infants heart diseases [1, 2]. Although CPB/DHCA enables repair of cardiac defects, it carries the risk of neurologic injury, with the manifestations including seizures, impaired cognition, cerebral palsy, etc. Therefore, the study of relevant pathophysiological factors associated with the CPB/DHCA procedures has gained increasing attention in the management of surgery and development of more effective protection strategies [3].

CPB/DHCA models in large animals such as dogs, cows, sheep and pigs have been established successfully in experimental studies [4, 5]. Recently, a CPB/DHCA model in rats was proposed, which does not require a full scale operating environment and reduces the difficulty for model implementation [6, 7]. There are several critical operation risks which might affect the neurologic recovery after CPB/DHCA, including the duration of arrest, duration of cooling and carbon dioxide tension during CPB before arrest, etc. [1]. Therefore, monitoring the physiologic changes during the surgery is critical in assessing the model. For example, electrical signals in brain such as electroencephalographic activity and somatosensory evoked potentials during recirculation after arrest were studied as indicators on neurophysiologic function [8, 9]. Cerebral blood flow (CBF) could also serve as an important physiological parameter and provides valuable hemodynamic information during the CPB/DHCA model [10]. It has been agreed that CBF variations during the CPB/DHCA surgery will offer useful insights into the cerebrovascular dynamics in the pathogenesis of brain cellular injury.

In this study, we are going to utilize a self-developed miniaturized head-mounted laser speckle imaging (LSI) system to monitor the real-time CBF changes during the CPB/DHCA surgery [11]. The spatiotemporal changes of CBF during the whole CPB/DHCA surgery were monitored and studied with the ultimate goal of finding useful indicators for the potential neurologic injury after surgery.

II. Materials and Methods

A. Animal Preparation

Five male Sprague-Dawley rats (450 ± 50g) were prepared for CPB/DHCA surgery. Anesthesia was induced with 5% Isoflurane and maintained with 1.0~1.5% Isoflurane in 2/3 N_2O and 1/3 O_2 [12]. The body temperature was maintained at 37.0 ± 0.5 °C before the CPB/DHCA surgery with a heating

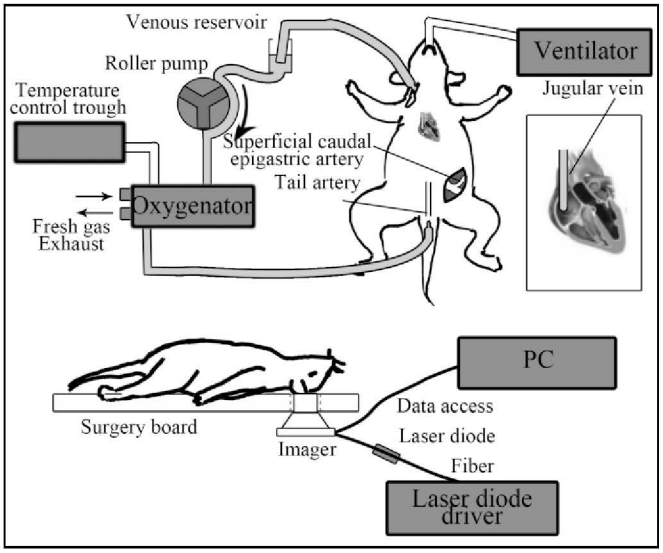

Figure 1 Overview of CPB/DHCA system (a) and Real-time CBF monitoring system (b).

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pad. The animals were then fixed in a stereotaxic frame (Benchmark Deluxe™, MyNeurolab.com, St. Louis, Missouri) for CPB/DHCA surgery preparation. A midline incision was made on the scalp, and tissues were cleaned to expose the surface of the skull with a scalpel. All procedures were performed under standard sterile precautions. A 7 mm × 7 mm window centered at 3.5 mm posterior to the bregma over the cortex was polished with a high-speed dental drill (Fine Science Tools Inc., North Vancouver, Canada) until the cortical vessels were clearly visible. Then a cylinder base was fixed onto the skull over the thinned area using reinforced glass ionomer cements (Dental Materials Factory of Shanghai Medical Instruments Co., Shanghai, China). After cement hardening, the cortex surface can be imaged during the entire experiment by connecting an LSI imager to the cylinder base [13].

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.

B. Imaging Procedure

Laser speckle imaging (LSI), as a full-field optical imaging technique, can provide CBF information in rats with high spatiotemporal resolution [11]. The laser speckle images (640 × 640 pixels) were acquired at 40 fps (exposure time, 5 ms) with a full-field cerebral blood flow imager (RatCap-3, Dolphin BioTech Ltd., Shanghai, China) over the skull illuminated by a 780-nm laser diode (L780P010, Thorlabs) during the entire experiment [13].

C. CPB Circuit Preparation

A non-pulsatile roller pump provided power to drive blood flow in the sterile silicone tubes (inner diameter, 1.6 mm, Tygon®, Cole-Parmer Instrument Co., Vernon Hills) of CPB circuit, as described previously [14]. The circuit flow rate was set to 120 mL·kg^{-1}·min^{-1}. Oxygenation of the circuit flow was controlled by a membrane oxygenator with a surface area of 0.1 m^2. The temperature of the circuit flow was controlled by a temperature control trough attached to the oxygenator. The total prime volume of the circuit was 20 mL, with 8 mL in the oxygenator [15].

D. DHCA Surgical Procedure

After endotracheal intubation, the animals were ventilated mechanically with 100% oxygen. The circuit flow originated from venous inflow through a 14-gauge (14-G) i.v. catheter, which was inserted and advanced about 3.5 cm until the cannula tip was placed near the junction of the superior vena cava and right atrium. The right common jugular vein was encircled with suture to avoid backflow. The oxygenated circuit flow was pumped back into circulation of the rat as arterial outflow in tail artery, which was encircled with suture and cannulated with the 14-G i.v. catheter. Fig. 1 shows the overview for the experimental setup. After baseline images were captured, the rat was cooled to a rectal temperature of 16°C to 18°C using surface cooling. CPB and ventilation were discontinued for circulatory arrest once the target temperature was reached. Then the rat was ventilated and actively warmed by the temperature control trough until the rectal temperature reached 37 °C. Animals received 150 IU i.v. heparin for the placement of the intravascular catheter. Arterial blood pressure and blood gas as well as the blood oxygen saturation level were monitored during the whole surgery [16].

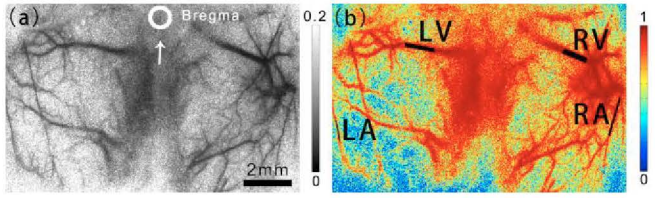

Figure 2 Laser speckle contrast image at the baseline of the CPB/DHCA surgery (a), and 4 ROIs are identified in its corresponding pseudo-color image: 2.4 mm to the midline in bilateral hemispherical veins and 5.1 mm to the midline in bilateral hemispherical arteries. Arrow indicates the anterior direction.

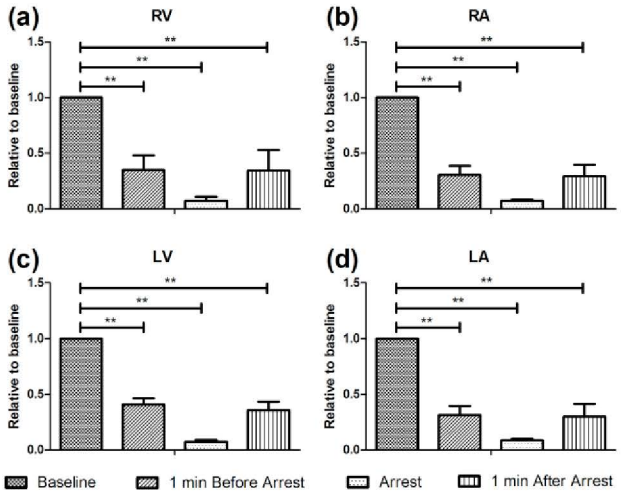

Figure 3 Relative blood flow velocity changes at four time points in vein and artery of bilateral hemispheres ($n = 5$). RV means the vein in right hemisphere, RA means the artery in right hemisphere, LV means the vein in left hemisphere and LA means the artery in left hemisphere.

E. Data Processing

The principle of LSI has been previously described in literature and will not be elaborated in details here [11, 17]. The contrast values for the CBF analysis were calculated every 320 registered images by the temporal laser speckle contrast analysis method [18]. It has been shown that the speckle contrast value K_s is inversely proportional to the CBF speed by

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

where T is the exposure time of the CCD and the auto-correlation time τ_c is inversely proportional to the CBF speed [17]. β is a constant parameter accounting for the loss of correlation [19]. All data were analyzed off-line with Matlab software (Mathworks Co., Ltd.). Four regions of interests (ROIs) were marked for quantitative CBF analysis, including the vein in right hemisphere, the artery in right hemisphere, the vein in left hemisphere and the artery in left hemisphere, which are shown in Fig. 2.

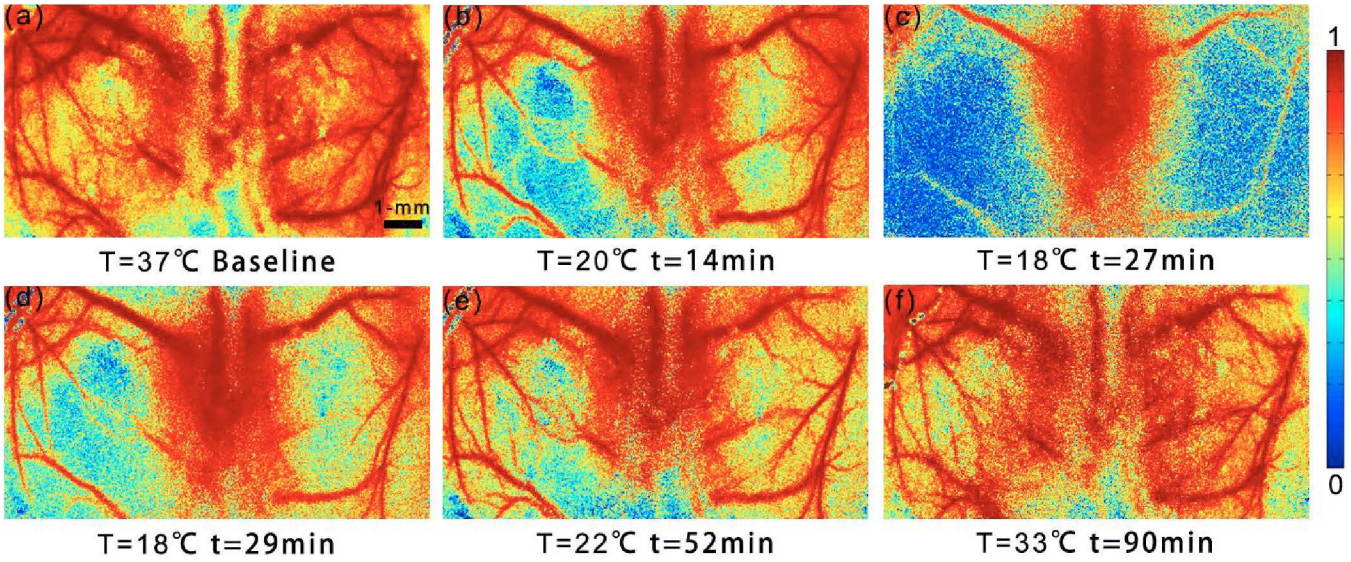

Figure 4 CBF images from the baseline to the end of the rewarming period. (a) Baseline, (b) During the cooling period, (c) During the arrest period, (d) At the beginning of the rewarming period, (e) In the middle of the rewarming period, (f) At the end of the rewarming period.

F. Statistics Analysis

Statistical comparison of CBF among baseline, 1 minute before arrest, during arrest, and 1 minute after arrest were carried out using a two-way analysis of variance (ANOVA) with SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). All data are presented as the means ± SD. Statistical significance was assumed when $P < 0.05$.

III. Results

A. Cerebral blood flow velocity variation

The CBF images were recorded continuously from baseline up to the end of the CPB/DHCA model. Fig. 4 shows CBF images in pseudo-color at different time points of the surgery. As it displays, CBF velocities declined in bilateral hemispheres during the cooling period. During the arrest period, CBF velocities in most areas dropped to nearly zero. Moreover, during the rewarming period, CBF velocities increased and returned to baseline level at the end.

We calculated the relative CBF velocities normalized over baseline level in the vein and artery of bilateral hemispheres. Fig. 3 shows the relative CBF at four different time points ($n = 5$). At 1 minute before the arrest, the CBF in the vein and the artery of right hemisphere decreased to 35.1% ± 11.5% ($P < 0.05$) and 30.4% ± 16.6% ($P < 0.05$) of baseline, and in the left hemisphere it decreased to 40.7% ± 11.5% ($P < 0.05$) and 31.5% ± 15.7% ($P < 0.05$) in vein and artery, respectively. At the time of arrest, the CBF in the vein and artery of right hemisphere further dropped to 7.5% ± 3.1% ($P < 0.05$) and 7.5% ± 2.0% ($P < 0.05$) of baseline, while the CBF in the vein and artery of left hemisphere decreased to 7.3% ± 3.2% ($P < 0.05$) and 8.6% ± 2.8% ($P < 0.05$), respectively. At 1 minute after the arrest, the CBF in the vein and artery of right hemisphere increased to 34.6% ±16.2% ($P < 0.05$) and 29.4% ± 20.5% ($P < 0.05$) of baseline, and the CBFs of left hemisphere raised to 35.6% ± 15.5% ($P < 0.05$) and 29.9% ±23.1% ($P < 0.05$).

Moreover, after taking a closer look at the CBF variations in the four regions during the whole cooling period, we'd some interesting findings. Among the five rats which went through the surgery, three rats showed a similar CBF changes pattern, as shown in Fig. 5(a). At the beginning of the cooling period, the CBF velocities rose for a short moment and then vibrated back. The relative variations of CBF velocities were similar in different vessels. However, in two other rats, the relative CBF changes in veins were more dramatic than in

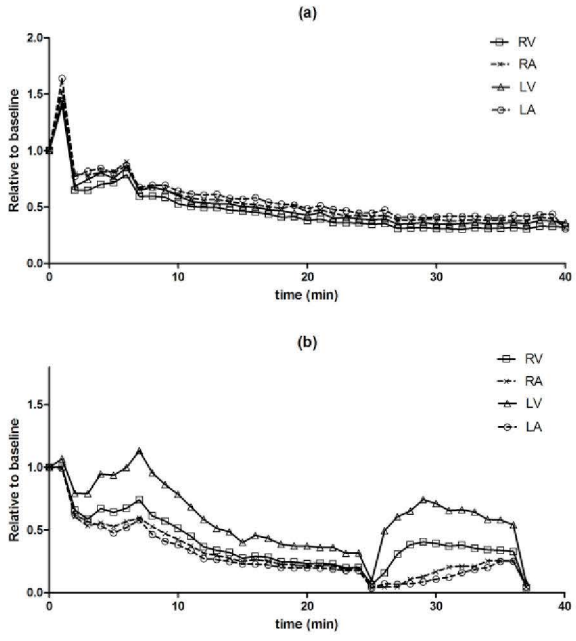

Figure 5 Two situations of relative CBFs in different vessels during cooling period (a, b). RV means the vein in right hemisphere, RA means the artery in right hemisphere, LV means the vein in left hemisphere and LA means the artery in left hemisphere.

arteries, with one shown in Fig. 5(b). More interestingly, both rats showed severe cerebral edema after the surgery, in contrast to other three.

B. The physiological variations between the rats

The whole processes of CPB/DHCA surgery in different rats were recorded. The records showed that the durations of cooling period ranged from 18 minutes to 50 minutes for all the rats. Also, the durations of rewarming period ranged from 31 minutes to 91 minutes, which are longer than the durations of cooling period. As we saw, to achieve the same rectal temperature changes, the required time for each rat was quite different due to the physiological variations.

IV. Discussions and Conclusions

In this study, we acquired the real-time full-field CBF images during the CPB/DHCA rat model with a miniaturized head-mounted LSI system. The data were recorded continuously during the whole CPB/DHCA surgery. The CBF information was analyzed in different vessels and compared with the baseline at different time points. We found that the CBF velocities declined during the cooling period and further dropped to nearly zero at the time of arrest. During the rewarming period, the CBF velocities increased until up to the baseline level at the end of rewarming.

In the beginning of the cooling period, there was a jitter in the CBF velocity changes, which might be caused by the hypothermal neuroprotective effects [20]. Moreover, two rats showed more dramatic CBF variations in veins than in arteries, which also exhibited severe cerebral edema after the surgery. This might be related to the lack of hematocrit, and it was known that low hematocrit might worsen the final physical situation after the rewarming period [21]. Also, the durations of cooling and rewarming periods were different for each rat due to the physiological variations. Thus more experiments need to be carried out in future to further validate the relation between CBF changes during the CPB/DHCA model with potential neurologic injury after surgery.

Our preliminary observations provided useful spatio-temporal information in the CBF changes during the whole CPB/DHCA model. The self-developed LSI system worked well with real-time CBF monitoring during surgical operations. The real-time CBF images acquired during the CPB/DHCA surgery could add more insights into the operation and be utilized to improve the surgical protocols with the ultimate goal of reducing neurologic injury after surgery.

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