Oxyhaemoglobin Level Measured Using Near-Infrared Spectrometer Is Associated with Brain Mitochondrial Dysfunction After Cardiac Arrest in Rats

Yu Okuma, Koichiro Shinozaki, Tsukasa Yagi, Kei Hayashida, Tomoaki Aoki, Tai Yin, Takeyuki Kiguchi, Taku Iwami, and Lance B. Becker

Abstract

Cerebral blood oxygenation (CBO), measured using near-infrared spectroscopy (NIRS), can play an important role in post-cardiac arrest (CA) care as this emerging technology allows for noninvasive real-time monitoring of the dynamic changes of tissue oxygenation. We recently reported that oxyhaemoglobin (oxy-Hb), measured using NIRS, may be used to evaluate the quality of chest compressions by monitoring the brain tissue oxygenation, which is a critical component for successful resuscitation. Mitochondria are the key to understanding the pathophysiology of post-CA oxygen metabolism. In this study, we focused on mitochondrial dysfunction, aiming to explore its association with CBO parameters such as oxy-Hb and deoxyhaemoglobin (deoxy-Hb) or tissue oxygenation index (TOI). Male Sprague-Dawley rats were used in the study. We applied NIRS between the nasion and the upper cervical spine. Following 10 min of CA, the rats underwent cardiopulmonary resuscitation (CPR) with a bolus injection of 20 μg/kg epinephrine. At 10 and 20 min after CPR, brain, and kidney tissues were collected. We isolated mitochondria from these tissues and evaluated the association between CBO and mitochondrial oxygen consumption ratios. There were no significant differences in the mitochondrial yields (10 vs. 20 min after resuscitation: brain, 1.33 ± 0.68 vs. 1.30 ± 0.75 mg/g; kidney, 19.5 ± 3.2 vs. 16.9 ± 5.3 mg/g, respectively). State 3 mitochondrial oxygen consumption rates, known as ADP-stimulated respiration, demonstrated a significant difference at 10 vs. 20 min after CPR (brain, 170 ± 26 vs. 115 ± 17 nmol/min/mg protein; kidney, 170 ± 20 vs. 130 ± 16 nmol/min/mg protein, respectively), whereas there was no significant difference in ADP non-dependent state 4 oxygen consumption rates (brain, 34.0 ± 6.7 vs. 31.8 ± 10 nmol/min/mg protein; kidney, 29.8 ± 4.8 vs. 21.0 ± 2.6 nmol/min/mg protein, respectively). Consequently,

Y. Okuma (✉)
Feinstein Institute for Medical Research, Northwell Health System, Great Neck, NY, USA

Department of Neurological Surgery, Sonoda Daiichi Hospital, Tokyo, Japan

Department of Neurological Surgery, Fukuyama City Hospital, Fukuyama, Japan

K. Shinozaki · T. Yagi · K. Hayashida · T. Aoki · T. Yin · L. B. Becker
Feinstein Institute for Medical Research, Northwell Health System, Great Neck, NY, USA

T. Kiguchi · T. Iwami
Kyoto University Health Service, Kyoto, Japan

F. Scholkmann et al. (eds.), *Oxygen Transport to Tissue XLIII*, Advances in Experimental Medicine and Biology 1395, https://doi.org/10.1007/978-3-031-14190-4_63

the respiratory control ratio (RCR = state 3/state 4) showed a significant difference over time, but this was only noted in the brain (brain, 5.0 ± 0.29 vs. 3.8 ± 0.64; kidney, 5.8 ± 0.53 vs. 6.2 ± 0.25 nmol/min/mg protein, respectively). The oxy-Hb levels had a dynamic change after resuscitation, and they had a significant association with the RCR of the brain mitochondria ($r = 0.8311$, $p = 0.0102$), whereas deoxy-Hb and TOI did not ($r = -0.1252$, $p = 0.7677$; $r = 0.4186$, $p = 0.302$, respectively). The RCRs of the kidney mitochondria did not have a significant association with CBO (oxy-Hb, $r = -0.1087$, $p = 0.7977$; deoxy-Hb, $r = 0.1565$, $p = 0.7113$; TOI, $r = -0.1687$, $p = 0.6896$, respectively). The brain mitochondrial respiratory dysfunction occurred over time, and it was seen at the time points between 10 and 20 min after CPR. The oxy-Hb level was associated with brain mitochondrial dysfunction during the early post-resuscitation period.

Keywords

NIRS · Cerebral blood oxygenation · Cardiopulmonary resuscitation · Tissue oxygenation index · Mitochondria

1 Introduction

Recent guidelines for cardiopulmonary resuscitation (CPR) state that high-quality CPR plays a key role in improving patient survival from cardiac arrest (CA) [1]. Previously, we introduced that the oxyhaemoglobin (oxy-Hb) levels between CA and during CPR have the potential to evaluate the quality of CPR in a rat CA model [2]. We also showed that near-infrared spectroscopy (NIRS) identified a decrease in oxy-Hb after the return of spontaneous circulation (ROSC), and this result has the potential to aid understanding of the mechanism of how and why adrenaline interferes with the improvement of neurological outcomes post-CA [3]. In this study, we focused on the relationship between mitochondrial function and cerebral blood oxygenation (CBO) parameters, including oxy-Hb, after CA.

2 Methods

2.1 Animal Preparation

The Institutional Animal Care and Use Committees of the Feinstein Institutes for Medical Research approved the study protocol. The details of the methods for a rat asphyxia CA model have been described previously [4, 5]. Briefly, adult male Sprague-Dawley rats (450–550 g, Charles River Laboratories) were anaesthetised with 4% isoflurane (Isosthesia, Butler-Schein AHS) and intubated with a 14-gage angio catheter (Surflo, Terumo Medical Corporation). Anaesthesia was maintained with isoflurane 2% at a fraction of inspired O_2 (FiO_2) of 0.3 under mechanical ventilation. The left femoral artery was cannulated (sterile polyethylene-50 catheter inserted for 20 mm) for continuous arterial pressure monitoring. The oesophageal temperature was maintained at 36.5 ± 1.0 °C during the surgical procedure. The left femoral vein was cannulated with a polyethylene-50 catheter, which was advanced into the inferior vena cava for drug infusion. We attached a NIRS (NIRO-200NX, Hamamatsu Photonics, Japan) from the nasion to the upper cervical spine of the rats. The distance between the emission and the detection probes was 3 cm. We examined the mean arterial pressure (MAP) and oxy/deoxy-Hb and tissue oxygenation index (TOI). The NIRS device recorded the oxygen saturation level (TOI) and the changes in concentration of oxy-Hb and deoxy-Hb in real-time (100 Hz). Data averaged every 20 s were used. After instrumentation setup, the neuromuscular blockade was achieved by slow intravenous administration of 2 mg/kg of vecuronium bromide (Hospira, USA). Asphyxia was induced in the rats by switching off the ventilator, and CA normally occurred 3–4 min after asphyxia started. We defined CA as a MAP below 20 mmHg; CA was completely untreated for 10 min after asphyxia. Mechanical ventilation was restarted at a FiO_2 of 1.0, and manual CPR

was delivered to the CA animals. Chest compressions were performed at a rate of 240–300 per min. At 30 s after the beginning of CPR, a 20 μg/kg bolus of adrenaline was given to the animals through the venous catheter. Following ROSC, to evaluate the brain mitochondrial dysfunction in conjunction with the measurement of CBO, we sacrificed the rats and collected the tissues at two-time points: 10 min and 20 min after CPR. Concerning CBO, we calculated the delta, which was the difference between the current average and the average of the end of the asphyxia period regarded as a baseline.

2.2 Isolation of Mitochondria and Evaluation of Mitochondrial Respiratory Function

All operations were performed at 4 °C. Harvested tissues were immediately placed in a mitochondrial isolation buffer (MESH) composed (in mM) of 210 mannitol, 70 sucrose, 10 Hepes, and 0.2 EGTA at pH 7.3 with 0.2% w/v fatty acid free-BSA (MESH-BSA). The tissue was blot-dried on filter paper, weighed, and placed in MESH-BSA. Low-speed centrifugation was set at 5600 × g for 1 min, and high-speed centrifugation was set at 12,000 × g for 6 min. Finally, the mitochondrial yields were expressed as mg mitochondrial protein/g tissue. For mitochondria isolation, the tissues were diluted in MESH-BSA for homogenisation. The homogenate was centrifuged at low speed, and the supernatant was collected. This supernatant was then centrifuged at high speed. The supernatant was gently decanted with pipettes without disturbing the mitochondria pellet. Finally, the pellet was resuspended in MESH without BSA and centrifuged at high speed. The mitochondria pellet was suspended, and the mitochondrial concentration was determined by the BCA assay using BSA as the standard. Concerning the method of measuring oxygen consumption rate (OCR), we used a Clark-type oxygen electrode (Strathkelvin, Motherwell, UK). Mitochondria were assayed in an assay buffer containing 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM KH_2PO_4, and 1 mg defatted BSA/mL at pH 7.4. ADP-dependent (state 3), ADP-limited (state 4), and DNP-dependent (uncoupled) OCR were measured at 30 °C in 150 μL of the mitochondrial suspension (0.5 mg/mL) using glutamate + malate as substrates. Rates of substrate oxidation were expressed as nano-atoms oxygen consumed/minute/mg mitochondrial protein [6, 7].

2.3 Statistical Analysis

Data are presented as mean and standard deviation. The Mann–Whitney U test was used to compare variables between two independent groups. Other values were compared between the groups using the one-way analysis of variance with post-hoc analysis using Tukey's test. In the comparison of multiple parameters, correlation coefficient (r) values were collected. All statistical analyses were conducted using JMP (version 10.1 software: SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically significant.

3 Results

ROSC was achieved in all animals. First, we determined the mitochondrial respiratory function by comparing the 10-min post-CPR group with the 20-min post-CPR group. Regarding the yield of isolated tissues, there was no significant difference between the two groups (brain, 1.33 ± 0.68 and 1.30 ± 0.75 mg/g; kidney, 19.5 ± 3.2 and 16.9 ± 5.3 mg/g, respectively). Regarding state 3 OCR, there were significant differences in both brain and kidney tissues between the two groups (brain, 170 ± 26 and 115 ± 17 nmol/min/mg protein; kidney, 170 ± 20 and 130 ± 16 nmol/min/mg protein, respectively). Regarding state 4 OCR, there was no significant difference among the groups (brain, 34.0 ± 6.7 and 31.8 ± 10 nmol/min/mg protein; kidney, 29.8 ± 4.8 and 21.0 ± 2.6 nmol/min/mg protein, respectively). Regarding the respiratory control ratio (RCR), there was a significant difference in

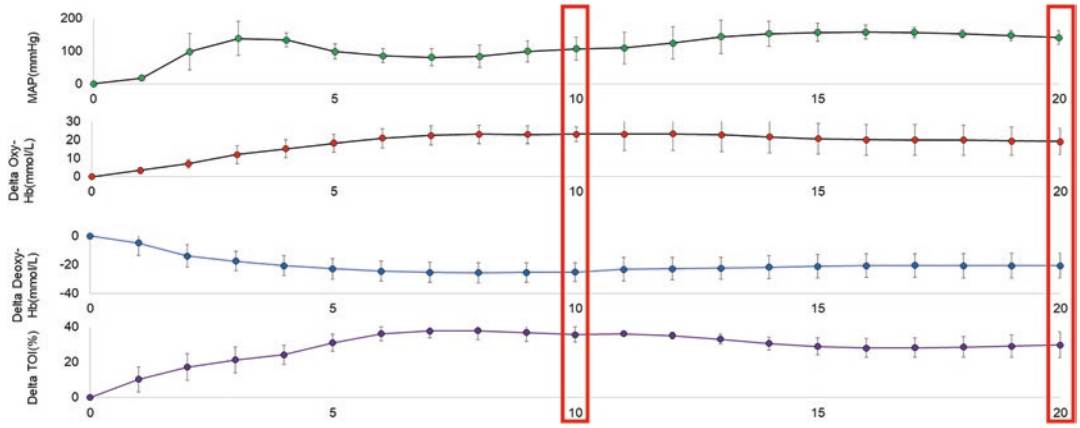

Fig. 1 The dynamic changes of the mean arterial pressure and differences in cerebral blood oxygenation, including oxyhaemoglobin and deoxyhaemoglobin and tissue oxygenation index, during the three types of chest compression. The delta is the difference between the current average and the average at the end of the asphyxia period, which is regarded as the baseline

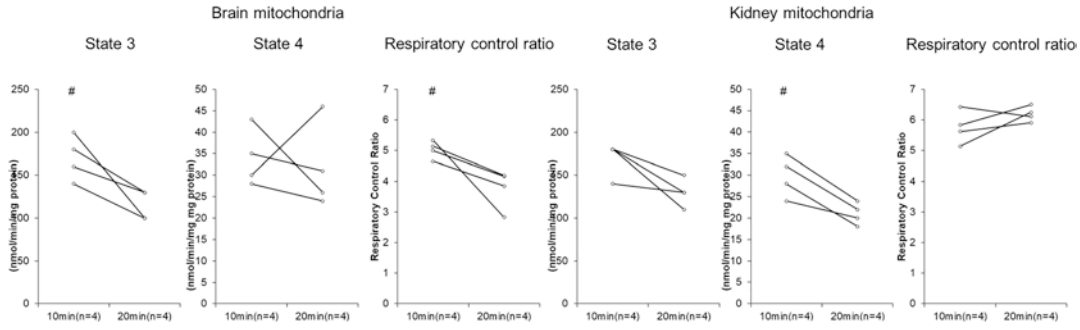

Fig. 2 The evaluation of mitochondrial respiratory function isolated in the brains and kidneys of the animal models. 10 min, 10-min-after-CPR; 20 min, 20-min-after-CPR. #P < 0.05 compared with the 20-min-after-CPR group

the brains between the two groups (brain, 5.0 ± 0.29 and 3.8 ± 0.64; kidney, 5.8 ± 0.53 and 6.2 ± 0.25 nmol/min/mg protein, respectively) (Fig. 1). We next evaluated the MAP and CBO by comparing the 10-min post-CPR group with the 20-min post-CPR group, which did not reveal any significant differences between the groups among all parameters (MAP, 107 ± 35 and 141 ± 21 mmHg, p = 0.107; delta oxy-Hb, 23.1 ± 4.0 and 19.1 ± 7.2 nmol/L, p = 0.552; delta deoxy-Hb, −25.4 ± 6.7 and −20.8 ± 9.0 nmol/L, p = 0.445; delta TOI, 35.8 ± 4.4 and 29.9 ± 7.3%, respectively) (Fig. 2). Finally, we examined the correlation between brain mitochondrial function and CBO. Concerning the brain mitochondria, only oxy-Hb showed a significant correlation with the RCR (r = 0.8311, p = 0.0102), whereas deoxy-Hb and TOI did not show a significant correlation with the RCR (r = −0.1252, p = 0.7677; r = 0.4186, p = 0.302, respectively) Concerning the kidney mitochondria, none of the CBO parameters showed a significant correlation with the RCR (oxy-Hb, r = −0.1087, p = 0.7977; deoxy-Hb, r = 0.1565, p = 0.7113; TOI, r = −0.1687, p = 0.6896, respectively) (Table 1).

Table 1 Relationship between cerebral blood oxygenation (CBO), including oxyhaemoglobin (Oxy-Hb) and deoxyhaemoglobin (Deoxy-Hb), tissue oxygenation index (TOI), and brain mitochondrial function or kidney mitochondrial function, including state 3, state 4, and respiratory control ratio (RCR)

	CBO		Brain mitochondrial function			Kidney mitochondrial function		
	Deoxy-Hb (n = 8)	TOI (n = 8)	State3 (n = 8)	State4 (n = 8)	RCR (n = 8)	State3 (n = 8)	State4 (n = 8)	RCR (n = 8)
Oxy-Hb	−0.069	0.23	0.42	−0.37	**0.83***	0.70	0.59	−0.11
Deoxy-Hb		**−0.91***	−0.49	−0.51	−0.13	−0.12	−0.17	0.16
TOI			0.53	0.30	0.42	0.21	0.22	−0.17
State3				0.58	0.55		**0.94***	−0.41
State4					−0.35			−0.70

*$P < 0.05$

4 Discussion

This experiment reported here demonstrates that the dynamic change of oxy-Hb was associated with the RCR of the brain mitochondria but not that of the kidney. This is the first study of its kind that investigated the relationship between oxy-Hb and mitochondrial dysfunction after CPR. Our previous study has revealed that the values of oxy-Hb during the CPR phase could support the quality of CPR. Some studies have brought up the concept of evaluating brain mitochondrial dysfunction by using NIRS. One of the novel methods utilised CBO and brain cytochrome-c-oxidase [8], and NIRO was reported in a clinical study [9]. Utilising NIRS measurements is difficult due to the large variability of CBO between individuals. To adjust for differences between individuals, we standardised the values of CBO with itself during CA (asphyxiation). Previously, our study showed that the CBO, as detected by NIRS and MAP, had similar levels between 10 and 20 min after CPR. Thus, in this study, we determined whether NIRS could serve as a monitor for the ischemic-reperfusion acute phase 10 and 20 min after CPR. The present study has several limitations. The CBO and mitochondrial dysfunction should be compared not only at two-time points but also at additional time points, which may be realised in future studies. The extent to which ischemic and reperfusion injuries are involved also needs to be evaluated. The evaluation of mitochondrial dysfunction other than that from oxygen consumption measurements, or the evaluation of brain damage based on nonmitochondrial mechanisms, should also be conducted. For example, it might be helpful to measure mitochondrial enzyme activities such as citrate synthetase [10] to further evaluate the health status /quality of the isolated mitochondria at the two time points used in this study. Additionally, it is necessary to investigate whether the levels of oxy-Hb in other organs are correlated with the level of mitochondrial dysfunction in those organs.

5 Conclusion

Oxy-Hb has the potential to correlate with the brain mitochondrial dysfunction and Oxy-Hb may be a useful parameter of the post-CA syndrome.

References

1. Neumar RW, Shuster M, Callaway CW et al (2015) Part 1: Executive summary: 2015 American Heart Association guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation 132(18 Suppl 2):S315–S367
2. Okuma Y, Becker LB, Yagi T et al (2021) Evaluation of the quality of chest compression with oxyhemoglobin level by near-infrared spectroscopy in a rat asphyxia cardiac arrest model. Adv Exp Med Biol 1269:265–269
3. Okuma Y, Yagi T, Yin T et al (2021) Effect of adrenaline on cerebral blood oxygenation measured by NIRS in a rat asphyxia cardiac arrest model. Adv Exp Med Biol 1269:39–43
4. Okuma Y, Shinozaki K, Yagi T et al (2019) Combination of cardiac and thoracic pump theories in rodent cardiopulmonary resuscitation: a new method of three-side chest compression. Intensive Care Med Exp 7:62
5. Okuma Y, Aoki T, Miyara SJ et al (2021) The evaluation of pituitary damage associated with cardiac arrest: an experimental rodent model. Sci Rep 11:629
6. Kim J, Perales Villarroel JP, Zhang W et al (2016) The responses of tissues from the brain, heart, kidney, and liver to resuscitation following prolonged cardiac arrest by examining mitochondrial respiration in rats. Oxidative Med Cell Longev 2016:1–7
7. Okuma Y, Becker LB, Hayashida K et al (2021) Effects of post-resuscitation normoxic therapy on oxygen-sensitive oxidative stress in a rat model of cardiac arrest. J Am Heart Assoc 10:e018773
8. Wang X, Tian F, Reddy DD et al (2017) Up-regulation of cerebral cytochrome-c-oxidase and hemodynamics by transcranial infrared laser stimulation: a broadband near-infrared spectroscopy study. J Cereb Blood Flow Metab 37:3789–3802
9. Holper L, Lan MJ, Brown PJ et al (2019) Brain cytochrome-c-oxidase as a marker of mitochondrial function: a pilot study in major depression using NIRS. Depress Anxiety 36:766–779
10. Aoki T, Okuma Y, Becker LB et al (2021) Methodological issue of mitochondrial isolation in acute-injury rat model: asphyxia cardiac arrest and resuscitation. Front Med 8:448