

Extracorporeal Membrane Oxygenation Improving Survival and Alleviating Kidney Injury in a Swine Model of Cardiac Arrest Compared to Conventional Cardiopulmonary Resuscitation

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Abstract

Background: Acute kidney injury (AKI) frequently occurs in cardiopulmonary resuscitation patients. Studies comparing the effects of extracorporeal membrane oxygenation (ECMO) with conventional cardiopulmonary resuscitation (CCPR) on AKI were rare. This study aimed to compare the effects of ECMO with those of CCPR on survival rate and AKI and explore the underlying mechanisms in a swine model of cardiac arrest (CA).

Methods: Sixteen male pigs were treated with ventricular fibrillation to establish CA model and then underwent CCPR (CCPR group, $n = 8$) or ECMO during cardiopulmonary resuscitation (ECPR group, $n = 8$). The study endpoints were 6 h after return of spontaneous circulation (ROSC) or death. Serum and urine samples were collected at baseline and during the 6 h after ROSC. The biomarkers of AKI were detected by enzyme-linked immunosorbent assay. The apoptosis of renal tubular epithelial cells was discovered by transmission electron microscope (TEM) and terminal deoxynucleotidyl transferase dUTP nick end labeling assay. Apoptosis-related genes were detected by immune-staining and Western blotting. Data were compared by Student's *t*-test.

Results: All pigs in ECPR group were successfully resuscitated with a higher 6-h survival rate (8/8) compared to CCPR group (6/8). The expressions of AKI biomarkers including neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinase2 (TIMP2), insulin-like growth factor-binding protein 7 (IGFBP7), liver fatty acid-binding protein (LFABP), and kidney injury molecule1 (Kim-1) were all increased along with the time after ROSC in both groups and lower in ECPR group compared with CCPR group. Especially, products of urinary TIMP and IGFBP levels (TIMP*IGFBP) were significantly lower at ROSC4 ($0.58 \pm 0.10 \text{ ng}^2/\text{ml}^2$ vs. $1.18 \pm 0.38 \text{ ng}^2/\text{ml}^2$, $t = 4.33$, $P = 0.003$) and ROSC6 ($1.79 \pm 0.45 \text{ ng}^2/\text{ml}^2$ vs. $3.00 \pm 0.44 \text{ ng}^2/\text{ml}^2$, $t = 5.49$, $P < 0.001$); urinary LFABP was significantly lower at ROSC6 ($0.74 \pm 0.06 \text{ pg/ml}$ vs. $0.85 \pm 0.11 \text{ pg/ml}$, $t = 2.41$, $P = 0.033$); and urinary Kim-1 was significantly lower at ROSC4 ($0.66 \pm 0.09 \text{ pg/ml}$ vs. $0.83 \pm 0.06 \text{ pg/ml}$, $t = 3.99$, $P = 0.002$) and ROSC6 ($0.73 \pm 0.12 \text{ pg/ml}$ vs. $0.89 \pm 0.08 \text{ pg/ml}$, $t = 2.82$, $P = 0.016$). Under light microscope and TEM, the morphological injuries in renal tissues were found to be improved in ECPR group. Moreover, apoptosis was also alleviated in ECPR group.

Conclusions: Compared with CCPR, ECMO improves survival rate and alleviates AKI in a swine model of CA. The mechanism of which might be via downregulating AKI biomarkers and apoptosis in kidney.

Key words: Acute Kidney Injury; Cardiac Arrest; Cardiopulmonary Resuscitation; Extracorporeal Membrane Oxygenation; Swine

INTRODUCTION

Cardiovascular disease is the first killer of the health worldwide.^[1] Extracorporeal membrane oxygenation (ECMO), an artificial extracorporeal system capable of providing temporary respiratory and circulatory support, has been widely used for the refractory cardiac and/or respiratory failure at present.^[2-4] Recent studies suggested

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Received: 10-04-2018 **Edited by:** Ning-Ning Wang
How to cite this article: Yuan XL, Li CS, Zhang Y, Ling JY, Zhang Q, Liang Y, Liu B, Zhao LX. Extracorporeal Membrane Oxygenation Improving Survival and Alleviating Kidney Injury in a Swine Model of Cardiac Arrest Compared to Conventional Cardiopulmonary Resuscitation. Chin Med J 2018;131:1840-8.

Access this article online

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DOI:
10.4103/0366-6999.237406

that ECMO therapy strategies can improve clinical outcomes in cardiac arrest (CA) patients compared with conventional cardiopulmonary resuscitation (CCPR).^[5-9]

Acute kidney injury (AKI) is one of the features of postresuscitation syndrome and can lead to acute renal failure.^[10] AKI occurrence is found to be frequent in patients undergoing ECMO and associated with development of multiorgan dysfunction and poor survival.^[11,12] In the critically ill patients treated with ECMO, the rate of renal replacement treatment (RRT) dependence ranges between 2% in bypass surgery patients and 65% in refractory postcardiotomy cardiogenic shock patients.^[13,14] Moreover, the need for RRT is considered as an independent predictor of mortality in patients treated with ECMO.^[15]

However, the studies comparing the effects of ECMO with CCPR on AKI were rare and the potential molecular mechanisms were not clear. Here, we established a swine model of refractory CA to compare the effects and investigate the underlying mechanisms.

METHODS

Ethical approval

This study was approved by the Capital Medical University Institutional Animal Care Committee and the Beijing Chao-Yang Hospital Affiliated to the Capital Medical University Animal Care and Use Committee.

Animal preparation

Sixteen male pigs, with a mean body weight of 35.13 ± 5.57 kg, were randomly divided into groups: CCPR group (treated with CCPR after CA, $n = 8$) and ECPR group (treated with ECMO during cardiopulmonary resuscitation after CA, $n = 8$). All the pigs were fasted overnight except for free access to water and were initially sedated by intramuscular injection with 10.0 mg/kg ketamine and 0.5 mg/kg midazolam; subsequently, anesthesia was induced by ear vein injection with a bolus dose of 2.0 mg/kg propofol. Then, the swine were supinely secured on the operating table for the following procedures. To keep sufficient anesthetic and analgesic depth, boluses of anesthesia (propofol 1.0 mg/kg) and analgesics (fentanyl, 4.0 μ g/kg) were then administered intravenously. In the case of the desired depth, propofol (9.0 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and fentanyl (1.0 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) were given to maintain the level of anesthesia and analgesics according to physiological parameters, corneal and palpebral reflexes, and spontaneous movement. There was no difference in body weight and other characteristics, including the extra doses of propofol and fentanyl administered during the preparatory phase between CCPR and ECPR groups. At the conclusion of the experiment, potassium chloride (2.0 mEq/kg), in conjunction with general anesthesia, was used to euthanize the animals. The anesthetized animals were incubated with a 6.5-mm cuffed endotracheal tube into the trachea via a tracheotomy, and then animals were mechanically ventilated with a volume-controlled ventilator (Servo 900 c; Siemens, Berlin, Germany) at a tidal volume of 15.0 ml/kg and a

respiratory frequency of 12–20/min with the inspired oxygen fraction of 35% in the beginning. The aim of end-tidal partial pressure of carbon dioxide which was monitored with an in-line infrared capnography (CO2SMOplus monitor; Respirationics Inc., Murrysville, PA) between 35 and 40 mmHg can be achieved by adjusting respiratory frequency. Adequate baseline ventilation was confirmed by arterial blood gas (ABL80; Radiometer, Copenhagen, Denmark).

We placed three surface electrodes configured to a standard lead II electrocardiogram (ECG) which was recorded with a multichannel physiologic recorder (BL-420 F Data Acquisition and Analysis System; Chengdu TME Technology Co. Ltd., Sichuan, China) on the surface of the skin preparation.

We placed a 5 Fr pacing catheter in the right external jugular vein into the right ventricle to induce ventricular fibrillation (VF) and to maintain intravenous fluid therapy. The left femoral artery was dissected to insert a fluid-filled catheter (5 Fr; Terumo, Tokyo, Japan) into the aortic arch to measure mean arterial pressure (MAP) through a pressure transducer. A Swan-Ganz catheter (7 Fr; Edwards Life Sciences, Irvine, CA) was advanced from the left femoral vein and flow directed into the pulmonary artery to measure cardiac output (CO) by the thermodilution method. We placed a 14 Fr Cannula and Catheters-Venous Catheter (Dragon Laifu Medical Products Co. Ltd., Changzhou, Jiangsu, China) through the left internal jugular vein into the right atrium and a 12 Fr Artery Catheter through the right femoral artery into the ascending aorta for ECMO. A 16 Fr urine catheter connecting a total-care urine bag was inserted into the bladder by cystostomy to obtain the urine every hour. The operation was performed with aseptic surgical technique. All catheters were filled with heparinized normal saline (5.0 U/ml) to prevent clotting.

Hemodynamics was monitored by an HP monitor (M1165; Hewlett-Packard Co., Palo Alto, CA). Normal saline solution (5.0 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and colloidal fluid (5.0 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) were infused intraoperatively to replenish fluid losses.

Extracorporeal life support

The ECMO circuit consisted of one arterial input catheter and one venous output catheter, a Levitronix Centrimag console (Sarns/3M Healthcare, Ann Arbor, MI, USA), centrifugal pump head (MAQUET Cardiopulmonary AG, Germany), in line with Adult Microporous Membrane Oxygenator with Bioline Coating (MAQUET Holding B.V. and Co. KG, Rastatt, Germany) and a mechanical gas blender (Thoratec Corporation, Pleasanton, CA, USA).

The left internal jugular vein and the right femoral artery were exposed by cutdown. The ECMO circuit contained one 16 Fr venous outflow cannula which was placed in the left internal jugular vein and one 14 Fr arterial inflow cannula which was placed in the right femoral artery. The catheters were full of heparinized 0.9% saline and clamped; nevertheless, the heparin cannot be instilled into the animals before the induction of VF. The ECMO circuit was driven by a centrifugal pump head in line with an oxygenator.

The circuit was primed with colloid solution (1000 ml), preheparinized with 250 U/kg heparin, and had 50% oxygen air mix delivered to the oxygenator. We adjusted the oxygen/air flow repeatedly keep pO_2 and pCO_2 in blood leaving the oxygenator suitable. A temperature of $34^\circ C$ was maintained with water-circulating heat exchanger. VF was induced by a programmed electrical stimulation instrument (GY-600A; Kaifeng Huanan Instrument Co., Kaifeng, Henan, China) with mode S1S2 (300/200 ms), 40V, 8:1 proportion, and -10-ms step length. VF was confirmed by the presence of a characteristic electrocardiographic tracing and an immediate drop in arterial blood pressure.

Experimental protocol

Animals were prepared first according to the above description and ECMO circuit was placed. Then, the swine were permitted to balance for 30 min to achieve at a steady resting level and the baseline values were acquired. Next, VF was induced in all animals by a medical programmed stimulator (GY-600A; Kaifeng Huanan Instrument Co., Kaifeng, Henan, China) to the right ventricular apex until VF was verified by the ECG waveform, accompanied by a fall in arterial blood pressure. We ceased the mechanical ventilation if VF was induced successfully. The range of VF and the CPR durations was explored in the preliminary study. After 12 min untreated VF, CPR was performed manually for 2 min in both ECPR group and CCPR group according to the 2015 guideline for CPR. Subsequently, CPR was performed for 4 min followed by shock attempts in CCPR group and CPR combined with ECMO was performed for 4 min in ECPR group followed by shock attempts, respectively. After shock attempt, CPR was continuously performed in CCPR group and CPR combined with ECMO was continuously performed in ECPR group until the endpoints. Defibrillation was attempted using biphasic 4.0 J/kg using a Heartstart M3535A defibrillator (Philips Medical Systems, Bothell, WA, USA) for the first attempt between the right infraclavicular electrode and the apical electrode. If necessary for return of spontaneous circulation (ROSC), additional epinephrine (0.02 mg/kg) was injected into the right atrium by the 5 Fr pacing catheter in the right external jugular vein.

The endpoints were 6 h after ROSC or the death for all the animals in both groups. The ROSC was defined as MAP was beyond 60 mmHg for 20 min or a systolic blood pressure was over 80 mmHg. We would stop CPR in CCPR group until the ROSC, which was defined that MAP was beyond 60 mmHg for 20 min or a systolic blood pressure exceed 80 mmHg. Otherwise, CPR and defibrillation were operated alternately for CCPR group. For ECPR group, the flow rate of ECMO was adjusted from 3 L/min to 5 L/min to maintain the MAP beyond 60 mmHg. For the sake of pO_2 , 100–120 mmHg, oxygen flow was adjusted through the oxygenator. Animals were ventilated during the reperfusion of ECMO to keep end-tidal pCO_2 35–45 mmHg by adjusting respiratory frequency. Activated clotting time was maintained above 250 s.

Measurements

Hemodynamic

Hemodynamic parameters, including heart rate (HR), MAP, and CO, were monitored. HR was recorded by the standard lead II ECG. MAP was measured with a fluid-filled catheter advanced from the left femoral artery into the aortic arch through a pressure transducer with pulse indicator continuous cardiac output system. A Swan-Ganz catheter (7 Fr; Edwards Life Sciences, Irvine, CA, USA) was advanced from the left femoral vein and flow directed into the pulmonary artery to measure CO by the thermodilution method. All the values were recorded at baseline, at ROSC (ROSC0), and different time points after ROSC, including 1 h (ROSC1), 2 h (ROSC2), 3 h (ROSC3), 4 h (ROSC4), 5 h (ROSC5), and 6 h (ROSC6).

Serum and urine acute kidney injury biomarker

Blood and urine samples were collected in 0.109 mol/L trisodium citrate tubes (9:1 vol/vol) concomitantly at the following time points: at baseline, at ROSC0, ROSC1, ROSC2, ROSC3, ROSC4, ROSC5, and ROSC6. Blood samples were drawn from the left femoral cannulation. The primal 5 ml of each blood sample was thrown away owing to it might include heparinized normal saline. The volume of urine per hour was measured by the precious urine bag. The samples of blood and urine were immediately centrifuged at $1006 \times g$ for 10 min ($4^\circ C$), and then the supernatants were collected and stored at $-80^\circ C$ until analysis.

The enzyme-linked immunosorbent assay kits of creatinine, NGAL, tissue inhibitor of metalloproteinase2 (TIMP2), insulin-like growth factor-binding protein 7 (IGFBP7), liver fatty acid-binding protein (LFABP), and kidney injury molecule1 (Kim-1) were used for analysis according to the manufacture's instruction (Swine Cr/NGAL/LFABP/IGFBP7/Kim-1: Shanghai Renjie Biotechnology Company, Ltd., Shanghai, China; Swine TIMP2: Shanghai Sangon Biotechnology Company, Ltd., Shanghai, China).

Kidney histology examination

Samples from the kidney were fixed with 10% formalin, then dehydrated and embedded in paraffin, cut into section, stained with hematoxylin and eosin, and observed by an optical microscopy (CX41; Olympus, Tokyo, Japan).

Kidney ultrastructure examination

Fresh kidney tissues were cut into pieces ($1.0 \text{ mm} \times 1.0 \text{ mm} \times 1.0 \text{ mm}$), treated with 3% glutaraldehyde, then flushed with phosphate-buffered saline, fixed with 1% perosmic acid, and dehydrated with acetone. Ultrathin sections were placed on 200-mesh copper grids and double stained with 4% uranyl acetate and 0.2% lead citrate. Sections were examined under transmission electron microscopy (JEM-1010; JEOL, Tokyo, Japan). Ultrastructure of proximal tubular epithelial cells and mitochondria was observed.

Kidney immunostaining

Immunostaining was performed on the fixed kidney tissue slides using a standard protocol with primary antibodies,

including monoclonal anti-caspase 3, anti-caspase 9, and anti-Bcl2 antibodies in 1:10,000 dilution (Cell Signaling Technology; Danver, USA) and secondary horseradish peroxidase (HRP)-conjugated goat anti mouse antibody in 1:1,000 dilution. The staining results were observed by an optical microscopy (CX41; Olympus, Tokyo, Japan).

Terminal deoxynucleotidyl transferase dUTP nick end labeling assay

The apoptosis was examined using terminal deoxynucleotidyl transferase (TdT) dUTP nick end labeling (TUNEL) apoptosis detection kit (Millipore, USA) following the manufacturer's instructions. In brief, kidney tissue was fixed in 10% formalin, embedded in paraffin, and sliced into 5 μ m sections. The slides were deparaffinized, rehydrated, and incubated with 20.0 mg/ml proteinase K for 15 min. Endogenous peroxidase activity was inhibited using 3% hydrogen peroxide. Sections were then incubated with TdT enzyme at 37°C for 1 h. Staining was revealed using 3,3-diaminobenzidine chromogen and observed by an optical microscopy (CX41; Olympus, Tokyo, Japan).

Western blotting

For Western blot analysis, an equal amount of protein (35 μ g) from each tissue sample was separated on a 12% sodium dodecyl sulfate-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. The membranes were blocked in 5% nonfat milk at room temperature for 2 h to prevent nonspecific binding and then incubated at 4°C overnight with primary antibodies which were the same as immunostaining and used at 1:1000 dilution. Then, the membranes were incubated with HRP-conjugated secondary antibody at 1:2000 dilution for 2 h at room temperature and visualized using enhanced chemiluminescence reagents. Tubulin was used as the internal control of protein loading to normalize each sample.

Statistical analysis

All data statistics were performed by SPSS 19.0 software (IBM, NY, USA). Data were presented as the mean \pm standard deviation (SD). Comparisons were performed by Student's *t*-test between ECPR and CCPR groups. Survival after ROSC in different groups was investigated by Kaplan-Meier Curves. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Rate of survivals

All the animals in both ECMO and CCPR groups achieved ROSC. During the following 6 h observation after ROSC, 8/8 swine survived in ECMO groups while 6/8 swine survived in CCPR group [Figure 1a]. Rescue time for ROSC was also compared and that of ECMO groups was less than CCPR group [Figure 1b].

Hemodynamics

There was no difference of baseline HR, CO, and MAP between ECMO group and CCPR group. HR decreased along with the time after ROSC in both ECMO and CCPR

groups while CO increased in both groups [Figure 2a and 2b]. However, at each time point, especially at ROSC0, ROSC1, and ROSC2, the HR (ROSC0: 126.75 ± 7.48 beats/min vs. 161.25 ± 12.63 beats/min, $t = 6.65$, $P < 0.001$; ROSC1: 120.25 ± 12.02 beats/min vs. 149.50 ± 14.01 beats/min, $t = 4.48$, $P = 0.001$; ROSC2: 118.50 ± 9.72 beats/min vs. 144.00 ± 13.35 beats/min, $t = 4.37$, $P = 0.001$) and CO (ROSC0: 1.91 ± 0.36 ml·kg⁻¹·h⁻¹ vs. 2.93 ± 0.17 ml·kg⁻¹·h⁻¹, $t = 7.19$, $P < 0.001$; ROSC1: 2.30 ± 0.42 ml·kg⁻¹·h⁻¹ vs. 3.19 ± 0.41 ml·kg⁻¹·h⁻¹, $t = 4.34$, $P = 0.001$; ROSC2: 2.37 ± 0.43 ml·kg⁻¹·h⁻¹ vs. 3.23 ± 0.39 ml·kg⁻¹·h⁻¹, $t = 4.20$, $P = 0.001$) were lower in CCPR group compared to ECPR group. Moreover, MAP decreased in the CCPR group and increased in ECPR group [Figure 2c].

Serum creatinine and urine outputs

Serum creatinine and urine outputs are the most sensitive signature of renal function. In our study, we found that creatinine at different time points did not differ significantly between CCPR group and ECPR group [Figure 3a]. Furthermore, no difference of urine output was observed between ECPR group and CCPR group except at ROSC5 and ROSC6 that urine outputs were significantly increased in ECPR group [Figure 3b].

Serum and urine acute kidney injury biomarkers

ROSC6 was the peak time during our observation time for the expressions of urinary TIMP and IGFBP in both ECPR and CCPR groups. Furthermore, the increase was rapid from 4 to 6 h after ROSC. Products of urine TIMP and IGFBP levels (TIMP*IGFBP) at every time point, especially ROSC4 and ROSC6, in ECPR group were significantly lower than that in CCPR group (ROSC4: 0.58 ± 0.10 ng²/ml² vs. 1.18 ± 0.38 ng²/ml², $t = 4.33$, $P = 0.003$; ROSC6: 1.79 ± 0.45 ng²/ml² vs. 3.00 ± 0.44 ng²/ml², $t = 5.49$, $P < 0.001$) [Figure 4a].

As shown in Figure 4b, urine LFABP concentrations increased gradually. It was lower in ECPR group compared with CCPR groups at all time points and the difference was significant at ROSC6 (0.74 ± 0.06 pg/ml vs. 0.85 ± 0.11 pg/ml, $t = 2.41$, $P = 0.033$). The urinary Kim-1 concentrations at baseline and ROSC0, ROSC1, and ROSC2 in ECPR groups were similar with that in CCPR groups. And, there was significant difference at ROSC4 (0.66 ± 0.09 pg/ml vs. 83 ± 0.06 pg/ml, $t = 3.99$, $P = 0.002$) and ROSC6 (0.73 ± 0.12 pg/ml vs. 0.89 ± 0.08 pg/ml, $t = 2.82$, $P = 0.016$) between the two groups [Figure 4c].

The serum and urinary NGAL levels exhibited similar variation trend after ROSC. However, at each time point, the NGAL concentrations were lower in ECPR group. Especially at ROSC4 and ROSC6, the NGAL significantly decreased compared to CCPR group [Figure 4d and 4e].

Renal histopathology examination

Histology of the kidney in the swine treated with ECPR was different from CCPR. Under optical microscopy, the glomeruli of both groups showed no change, but swollen

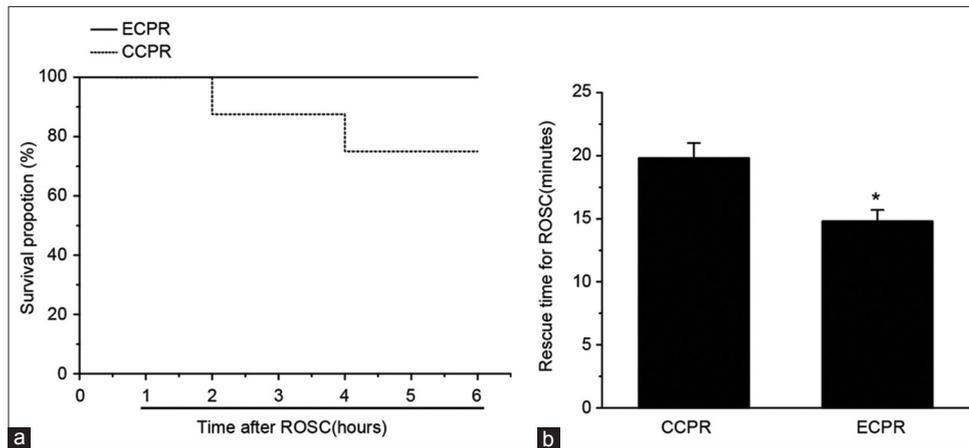


Figure 1: Comparison of survival rate and rescue time for ROSC in cardiac arrest swine undergoing ECPR ($n = 8$) or CCPR ($n = 8$). (a) Survival rate; (b) rescue time for ROSC. $*P < 0.05$. CCPR: Conventional cardiopulmonary resuscitation; ECPR: Extracorporeal cardiopulmonary resuscitation; ROSC: Return of spontaneous circulation.

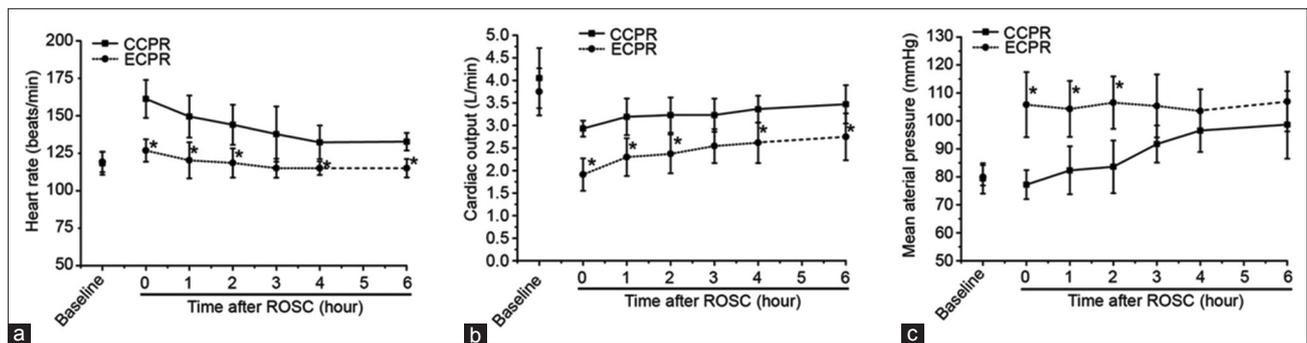


Figure 2: Comparison of hemodynamics in cardiac arrest swine undergoing ECPR ($n = 8$) or CCPR ($n = 8$). (a) Heart rate; (b) cardiac output; (c) mean arterial pressure. $*P < 0.05$. CCPR: Conventional cardiopulmonary resuscitation; ECPR: Extracorporeal cardiopulmonary resuscitation; ROSC: Return of spontaneous circulation.

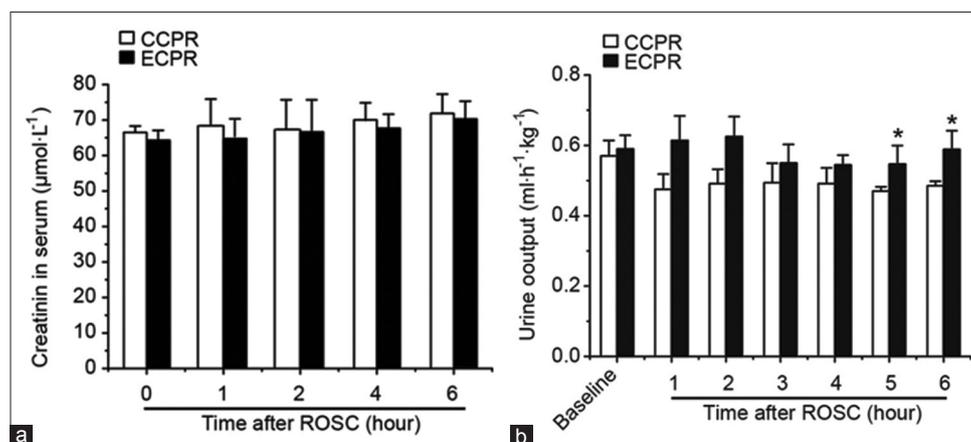


Figure 3: Comparison of serum creatinine and urine output at baseline and different time points after ROSC in cardiac arrest swine undergoing ECPR ($n = 8$) or CCPR ($n = 8$). (a) Serum creatinine; (b) urine output. $*P < 0.05$. CCPR: Conventional cardiopulmonary resuscitation; ECPR: Extracorporeal cardiopulmonary resuscitation; ROSC: Return of spontaneous circulation.

and balloon renal tubular epithelial cells were less in ECPR groups than that in CCPR group. There was no obvious edema in the renal interstitial fluid in both groups; nevertheless, a large number of inflammatory cells infiltrated in the renal interstitial areas in CCPR group whereas little was observed in ECPR group [Figure 5].

Ultrastructure of kidney in ECPR and CCPR groups was observed under electromicroscopy and characteristic morphological changes of apoptosis were observed in both groups. However, the changes including basement membrane degeneration, nuclear condensation, and nuclear cleavage to form nuclear fragments, i.e. apoptotic bodies, were much

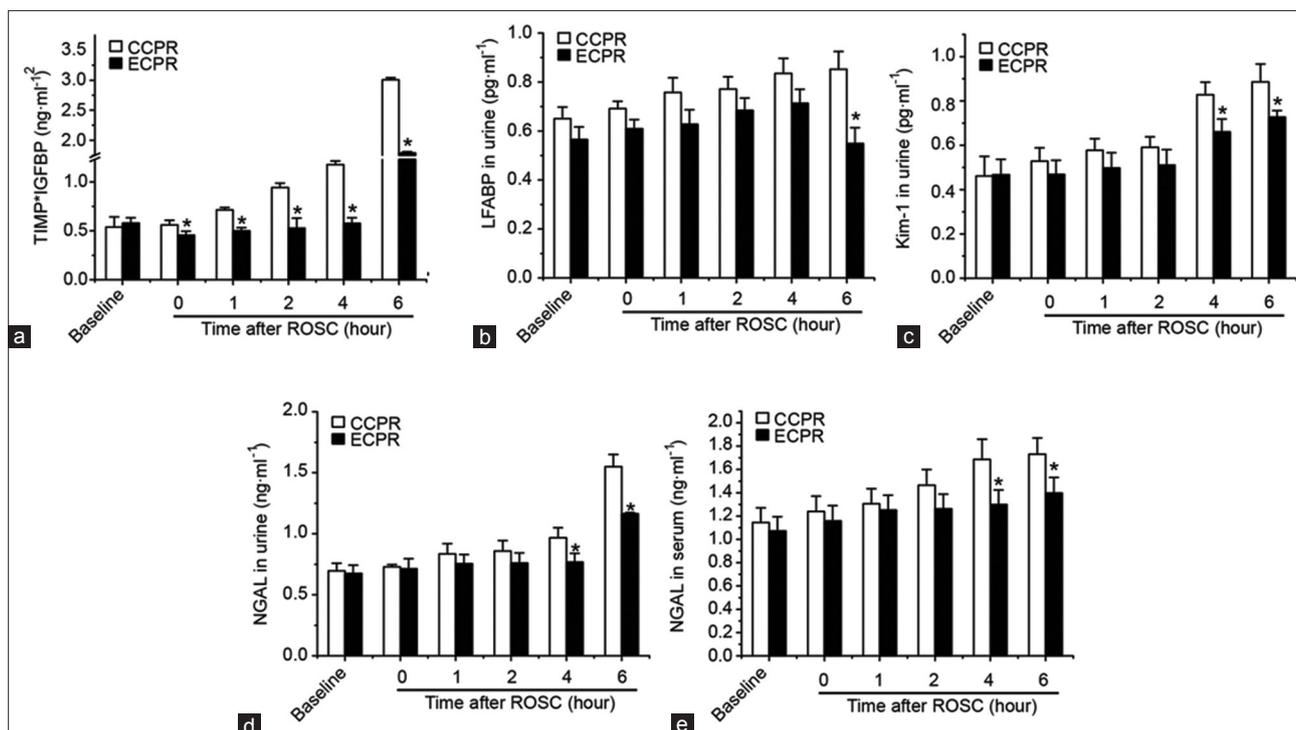


Figure 4: Comparison of serum or urine biomarkers at baseline and different time points after ROSC in cardiac arrest swine undergoing EPCR ($n = 8$) or CCPR ($n = 8$). (a) Urine TIMP*IGFBP; (b) urine LFABP; (c) urine Kim-1; (d) urine NGAL; (e) serum NGAL. * $P < 0.05$. CCPR: Conventional cardiopulmonary resuscitation; EPCR: Extracorporeal cardiopulmonary resuscitation; ROSC: Return of spontaneous circulation; TIMP: Tissue inhibitor of metalloproteinase 2; IGFBP7: Insulin-like growth factor-binding protein 7; LFABP: Liver fatty acid binding protein; Kim-1: Kidney injury molecule 1; NGAL: Neutrophil gelatinase-associated lipocalin.

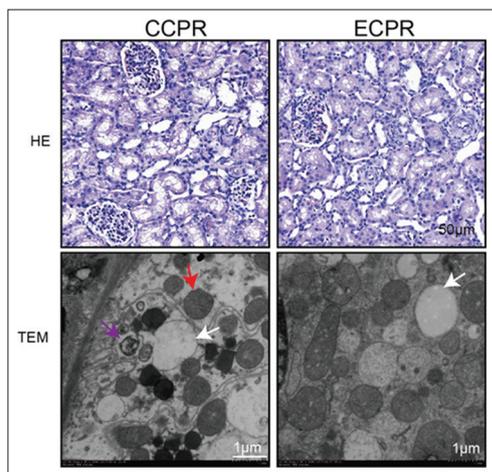


Figure 5: Representative images of hematoxylin and eosin staining and ultrastructure examination of the kidney tissues cardiac arrest swine undergoing EPCR ($n = 8$) or CCPR ($n = 8$). White arrow: Enlarged endoplasmic reticulum; Purple arrow: Phagocytic vacuole; Red arrow: Deformed mitochondria; CCPR: Conventional cardiopulmonary resuscitation; EPCR: Extracorporeal cardiopulmonary resuscitation; HE: Hematoxylin and eosin staining; TEM: Transmission electron microscope.

more serious in CCPR group compared to EPCR group. The mitochondria and endoplasmic reticulum swelling and lysosome and phagocytic vacuole increase in the damaged renal tubular epithelial cell were obvious in CCPR group and occasional in EPCR group [Figure 5].

Renal apoptosis

The apoptosis was first determined by TUNEL assay and the results suggested that apoptosis was decreased in EPCR groups compared with CCPR group. Then, BCL2 expression and cleavage of caspase 3 and 9 were detected by immunostaining. More BCL2 levels and less cleaved caspase 3 and 9 were observed in EPCR group [Figure 6a]. Furthermore, cleavage of caspase 3 and 9 was detected by Western blotting and quantified and normalized to internal control, Tubulin. Similar with immunostaining results, decreased levels of cleaved caspase 3 and 9 were observed in EPCR group [Figure 6b-6d].

DISCUSSION

The importance of chest compression and artificial ventilation is emphasized since the guidelines of resuscitation were first published in the 1970s.^[16] However, chest compressions can only deliver the 25% of blood flow. In addition, success rate of the resuscitation decreases along with the rescuers getting fatigued. Thus, the International Liaison Committee on Resuscitation guidelines from 2015 recommends ECMO for CA patients.^[17] In our study, CA animals treated with ECMO exhibited increased survival rate compared with CCPR. Preliminary clinical data showed that venoarterial EPCR should be considered first in some CA patients.^[18]

In our model, HR was lower significantly at every time point after ROSC in EPCR group than in CCPR group, and

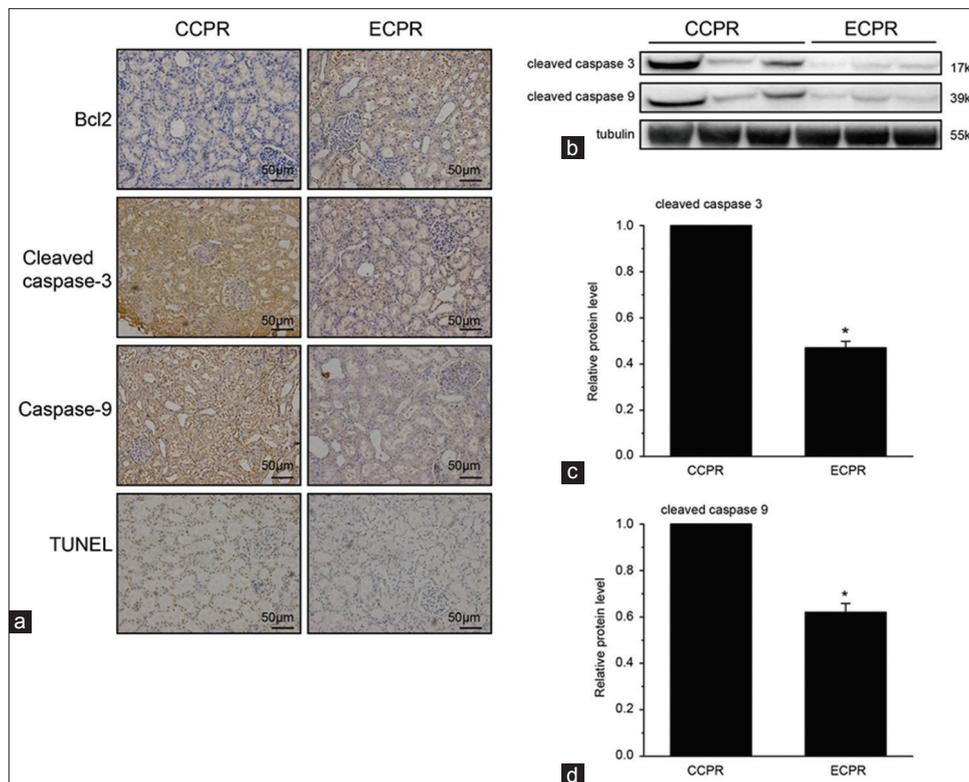


Figure 6: Apoptosis detection in cardiac arrest swine undergoing ECPR ($n = 8$) or CCPR ($n = 8$). Apoptosis-related proteins were detected by immunostaining and Western blotting. Apoptosis was also examined by TUNEL assay. (a) Representative images of immune-staining of Bcl2, cleaved caspase 3, and cleaved caspase 9 and TUNEL assay; cleaved caspase 3 and caspase 9 were detected by western blotting (b); and cleaved caspase 3 (c) and cleaved caspase 9 (d) levels were also quantified. * $P < 0.05$. CCPR: Conventional cardiopulmonary resuscitation; ECPR: Extracorporeal cardiopulmonary resuscitation; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling.

it is well known that slowing heart rate can help to reduce oxygen consumption. It is very important that spontaneous circulation returned rapidly to reduce the ischemic damage in tissues and organs during CPR after CA. ECPR can improve ROSC rate, ensure the adequate perfusion, and recover the oxygen metabolism. The improvement of systemic oxygenation with this technique makes the artificially oxygenated blood mixing with arterial blood and directly perfusing distal organs including the kidney. The VA-ECMO technique whose circuit is connected in parallel to the heart and lungs can provide both respiratory and cardiac support. Unlike cardiopulmonary bypass, VA-ECMO provides only about 80% of the predicted resting CO in the ideal setting; nevertheless, the rest of the perfusion is still provided by the heart.^[19] The total CO of the body is composed of native CO and VA-ECMO flow. Peripheral VA-ECMO decreases preload and increases the afterload of the left ventricular. In addition, animal studies have shown that the decline in preload leads to the decrease in left ventricular end-diastolic volume and pressure, thus promoting a better coronary perfusion pressure.^[20] MAP is directly related to the total aortic flow, the summation of the native CO, and the ECMO pump flow. The value of MAP should be kept above 60 mmHg for adequate organ perfusion. Hence, the decline of the preload leads to a lower CO in ECPR group than CCPR group. On the contrary, the pump flow and the increased left ventricular afterload contribute to the ascending of the MAP.

MAP in our ECPR group was higher than CCPR groups, suggesting an increased organ perfusion and reduced injury.

Creatinine and urine outputs are the current GOLD standard in diagnose of AKI according to the Kidney Disease Improving Global Outcomes clinical practice guideline. Other early AKI biomarkers, such as NGAL, KIM-1, L-FABP, and TIMP-2*IGFBP-7, have also been investigated and used to diagnose and treat AKI.^[21] Kaucsár *et al.*^[22] suggested that urinary NGAL concentrations and the ratio of urine to plasma NGAL are sensitive and specific biomarkers for subclinical AKI model. Adler *et al.*^[23] found that TIMP-2*IGFBP-7 can be used to predict AKI in out-of-hospital CA survivors. In our experiment, urine TIMP-2*IGFBP-7 in the ECPR group at every time point from baseline to ROSC6 were smaller than that in the CCPR group and serum and urinary NGAL were also found to be lower in ECPR group. These results were in concordance with animal phenotypes that AKI was slighter in ECPR group compared to CCPR group. Meanwhile, additional biomarkers might also be detected, such as serum lactate, which is associated with survival and neurological outcomes in CA patients undergoing ECMO and with AKI after CA.^[24,25]

It was well known that the proximal tubule epithelial cells are highly susceptible to apoptosis and injury in case of organ failure. Under physiological conditions, the proximal tubule cells are columnar in shape with highly polarized

basal-lateral and apical surface membrane domains that drive solute reabsorption or excretion. Extensive evidence suggests that detachment, dysfunction, and death of these cells are primarily responsible for the pathophysiological and clinical aspect of ischemic AKI.^[26] In the animal model of CA undergoing ECMO, the injured kidney showed the following changes: plasma membrane blebbing, loss of cell membrane asymmetry, diffuse ballooning, and deformation of the proximal tubule cell. In addition, a mass of “apoptotic bodies” appeared in CCPR group while it was almost invisible in ECMO group. Elmore^[27] reported cell detachment and DNA fragmentation during apoptosis; however, it was not observed in our study that might be due to the short observation time. Loss of tubular cells leads to unregulated paracellular diffusion of water, ions, and macromolecules, thereby causing increased back leak, which reduces glomerular filtration rate (GFR). The lumen is full of the damaged cells, the apical membrane material, and other cellular debris which aggregate a process mediated by the extrinsic RGD sequences within the extracellular domain of β -integrin to form casts, resulting in tubular obstruction.^[28] Together, loss of cell surface area and tubular obstruction decreases GFR. The rapid hemodynamic fluctuations in VA-ECMO cause renal microvascular blood flow to change and result in ischemia/reperfusion AKI. Alternatively, the restoration of renal blood flow with initiation of ECMO may also contribute to reperfusion of AKI.^[29] What we demonstrated in the study provided some morphological and molecular evidence of apoptosis induced by ischemia-reperfusion injury including caspase 3 and 9 activation and loss of intact Bcl2 that were alleviated by ECPR. Several limitations existed in our study. First, the physiology of our swine model might not reflect the real situation in CA patients and the results in our study should be confirmed in clinical. Second, we placed all the catheters of ECMO circuit in advance, but in the clinical work, there were some difficulty in cannulating. Third, our experimental endpoint was 6 h after ROSC and the observation duration was relative short. Finally, the sample size in the present study was limited and further study with larger sample size should be performed in future.

In a swine model of CA, we found that ECMO can improve survival rate and alleviate AKI compared with CCPR. The underlying mechanisms might be that ECMO can reduce the increase of AKI-related gene expression and apoptosis after CA.

Financial support and sponsorship

The study was supported by the National Natural Science Foundation of China (No. 81372025) and the 2015 Annual Special Cultivation and Development Project for the Technology Innovation Base of the Beijing Key Laboratory Cardiopulmonary Cerebral Resuscitation (No.Z151100001615056).

Conflicts of interest

There are no conflicts of interest.

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体外膜肺氧合较心肺复苏可以提高猪心脏骤停模型生存率并减轻肾损伤

摘要

背景：急性肾损伤常见于心肺复苏病人，目前比较体外膜肺氧合（ECMO）和常规心肺复苏（CCPR）对急性肾损伤影响的研究极少，同时其潜在分子机制也少有研究。本研究旨在利用心脏骤停猪模型比较ECMO和CCPR对其生存率和急性肾损伤的影响同时探究相关机制。

方法：16只猪经室颤建立心脏骤停模型，同时分成两组，ECPR组接受CCPR和ECMO治疗（n=8）；CCPR组只接受CCPR治疗（n=8）。研究终点为自主循环恢复（ROSC）6小时或死亡。收集基线及ROSC各时间点血清和尿液样本。急性肾损伤相关标志物经酶联免疫法检测。利用透射电镜和TUNEL方法观察肾小管上皮细胞凋亡水平。同时利用免疫染色和免疫印记检测凋亡相关基因。实验数据以平均值±标准差比较并用Student's *t*-检验进行比较。

结果：ECPR组均成功复苏，其6小时生存率（8/8；100%）高于CCPR组（6/8；75%）。包括TIMP、IGFBP、LFABP、KIM-1在内的急性肾损伤标志物在ECPR和CCPR组均随观察时间而增加。同时发现ECPR组各标志物水平均显著低于CCPR组。特别是ROSC4（ $0.58 \pm 0.10 \text{ ng}^2/\text{ml}^2$ vs. $1.18 \pm 0.38 \text{ ng}^2/\text{ml}^2$, $t=4.33$, $P=0.003$ ）和ROSC6（ $1.79 \pm 0.45 \text{ ng}^2/\text{ml}^2$ vs. $3.00 \pm 0.44 \text{ ng}^2/\text{ml}^2$, $t=5.49$, $P<0.001$ ）时间点的尿液TIMP与IGFBP水平之积；ROSC6时间点的尿液LFABP水平（ $0.74 \pm 0.06 \text{ pg/ml}$ vs. $0.85 \pm 0.11 \text{ pg/ml}$, $t=2.41$, $P=0.033$ ）；以及ROSC4（ $0.66 \pm 0.09 \text{ pg/ml}$ vs. $0.83 \pm 0.06 \text{ pg/ml}$, $t=3.99$, $P=0.002$ ）和ROSC6（ $0.73 \pm 0.12 \text{ pg/ml}$ vs. $0.89 \pm 0.08 \text{ pg/ml}$, $t=2.82$, $P=0.016$ ）时间点的尿液Kim-1水平。在光学显微镜和透射电镜下，均可观察到ECPR组肾组织形态损伤水平要低于CCPR组。ECPR组肾组织凋亡水平也得到了缓解。

结论：与常规心肺复苏相比，体外膜肺氧合可以提高心脏骤停生存率并缓解急性肾损伤。其分子机制可能是，体外膜肺氧合较常规心肺复苏降低了急性肾损伤标志物水平以及肾组织中的凋亡水平。