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A rodent model of emergency cardiopulmonary bypass resuscitation with different temperatures after asphyxial cardiac arrest[☆]

Fei Han^{a,b}, Manuel Boller^{a,c}, Wenhui Guo^a, Raina M. Merchant^a, Joshua W. Lampe^a, Thomas M. Smith^a, Lance B. Becker^{a,*}

^a Center for Resuscitation Science, Department of Emergency Medicine, University of Pennsylvania, Philadelphia, PA, United States

^b Department of Anesthesiology, The Third Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang, China

^c School of Veterinary Medicine, Department of Clinical Studies, University of Pennsylvania, Philadelphia, PA, United States

Abstract

Background—The use of emergency cardiopulmonary bypass (ECPB) resuscitation after cardiac arrest may offer hope for survival when standard ACLS therapies fail. However, whether cooling adds benefit to ECPB is unknown and we lack an ECPB rodent model for experimental studies. We sought to (a) develop a 72 h survival rodent model using ECPB to treat asphyxial cardiac arrest and (b) use this new model to evaluate early mild and moderate hypothermia versus normothermia during ECPB resuscitation.

Methods—After 8 min of normothermic asphyxia, three groups of rats were resuscitated with ECPB at 37°C (NORM), 34 °C (MILD) and 30 °C (MOD) for 1 h ($n = 10$ each). During the second resuscitation hour, ECPB was discontinued, ventilatory support was provided and body temperatures were maintained at 37 °C for NORM, 34 °C for MILD, and from 30 °C gradually up to 34 °C in 1 h for MOD animals. From hours 3 to 8, body temperature was maintained at 37 °C for NORM and 34 °C for MILD and MOD animals.

Results—All rats were initially resuscitated by ECPB. After 72 h, neurological outcome and survival in the MILD (60% survival) and MOD (80%) groups were significantly better than in the NORM (0%) group ($p < 0.05$). Overall performance recovery in the MOD group was best (vs. the NORM group), while the MILD group had an intermediate outcome.

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* Corresponding author at: Center for Resuscitation Science, Department of Emergency Medicine, School of Medicine, University of Pennsylvania, 125 S. 31st Street, TRL Building, Suite 1200, Philadelphia, PA 19104, United States. Tel.: +1 215 746 3581; fax: +1 215 746 1224. lance.becker@uphs.upenn.edu (L.B. Becker).

Conflict of interest

L.B. Becker has received honoraria and consulting fees from Philips Medical Systems, Gaymar Industries, Zoll Medical, Medtronic, and the NIH Data Safety Monitoring Board and Protocol Review Committee; holds patents related to hypothermia induction and reperfusion therapies; and holds equity and royalties from Cold Core Therapeutics, Inc. The remaining authors do not declare any relevant conflicts of interest.

Conclusions—A rodent model of ECPB is feasible and useful for resuscitation studies. The addition of early mild and moderate hypothermia to ECPB resuscitation significantly improves survival compared with normothermic ECPB in rats.

Keywords

Cardiopulmonary bypass; Heart arrest; Hypothermia; Cardiopulmonary resuscitation; Reperfusion

1. Introduction

Cardiovascular disease has remained a leading cause of death in the United States with many of these deaths occurring suddenly, and survival rates remain disappointing for cardiac arrest patients despite considerable efforts.^{1–3} Emergency cardiopulmonary bypass (ECPB) may be a life-saving advanced resuscitation strategy for patients who fail standard advanced cardiac life support (ACLS).^{4–9} ECPB consists of rapid initiation of femoral veno-arterial cardiopulmonary bypass (CPB), followed by extracorporeal circulatory support and oxygenation until restoration of effective cardiac output can be achieved. Several clinical reports have demonstrated its feasibility and have shown improved survival in both pediatric^{4–6} and adult^{7–9} patients. The reported overall survival rate is between 3.4% and 41%.^{6,8,10–12} Some groups have reported using therapeutic hypothermia as an adjunct with ECPB resuscitation^{5,7} while others have not utilized hypothermia.^{4,6} It remains unknown whether therapeutic hypothermia is of benefit with ECPB resuscitation. In contrast to advanced ECPB resuscitation, therapeutic hypothermia is accepted as an important intervention that provides benefit to patients who achieve stable return of spontaneous circulation (ROSC) following cardiac arrest with standard ACLS treatments. While many researchers and clinicians have extrapolated that hypothermia may prove helpful during resuscitation utilizing ECPB, there remain many unanswered questions about its possible benefit, including the optimal depth of hypothermia used with ECPB, the optimal timing of cooling and rewarming with ECPB, and the degree of protection that it provides. Studying ECPB is challenging because large animal experimental models are costly, and human studies with ECPB have significant barriers. We found no existing rodent model for the study of ECPB resuscitation after asphyxial cardiac arrest in the literature. For these reasons, we sought to (a) develop a rodent model of ECPB to treat asphyxial cardiac arrest and (b) use this new model to evaluate the benefit of early mild and moderate hypothermia versus normothermia during ECPB resuscitation.

2. Methods

The experimental protocol for the study was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Adult male Sprague–Dawley rats (weight 420–470 g, Charles River Production, Wilmington, MA, USA) were used for the study and were housed in a rodent facility under 12 h light–dark cycle with unrestricted access to food and water.

2.1. Developing a model of ECPB resuscitation in asphyxial cardiac arrest in rats

Our initial task was to modify an existing rodent model that used ECPB technology in the context of a lethal hemorrhage into an asphyxial cardiac arrest model. The existing model had been developed at the University of Pittsburgh specifically in rodent studies for emergency preservation and resuscitation which is defined as the temporary preservation of the viability of brain and organism for transport and repair during cardiac arrest followed by delayed resuscitation and one of our investigators (FH) had significant experience in this technique. All surgical procedures were performed with sterile techniques. Rats were anesthetized with 4% isoflurane in FiO_2 1.0 for 5 min in a transparent plexiglas chamber, orotracheally intubated with a 14-gauge intravenous catheter (B. Braun Medical Inc., Bethlehem, PA, USA) and mechanically ventilated with a tidal volume (TV) of 8 ml/kg which was adjusted to maintain an EtCO_2 between 35 and 45 mmHg (Micro-Capnometer, Columbus Instruments, Columbus, OH, USA), a respiratory rate (RR) of 40 min^{-1} and a positive end-expiratory pressure (PEEP) of 5 cm H_2O (Ventilator Model 683, Harvard Apparatus, Holliston, MA, USA). Anesthesia was maintained with 1–2% isoflurane in FiO_2 0.5 (N_2 0.5) to sustain a surgical depth of anesthesia. Temperatures were monitored using tympanic and rectal thermocouple probes and were maintained steadily at $37 \pm 0.5^\circ\text{C}$ with a water-based warming blanket and an incandescent heating lamp. The left femoral artery and vein were separately cannulated with polyethylene catheters (PE-50 tubing). The arterial cannula was used for continuous arterial blood pressure recording and blood gas assay sampling (i-STAT, Heska, East Windsor, NJ, USA). The venous cannula was positioned further in the chest and used for central venous pressure monitoring and for delivery of drugs or fluids. Body temperatures, ECG, SpO_2 , arterial and central venous pressure were continuously monitored and digitally recorded (Chart 5 for Windows, ADInstruments, Inc., Colorado Springs, CO, USA). One hundred and fifty units heparin was given through the left venous catheter. The right external jugular vein was cannulated with a custom modified six-hole 14-gauge cannula (BD Medical, Franklin Lakes, NJ, USA) and advanced to the vena cava for venous outflow to the CPB pump. An additional 150 U heparin was given via this catheter. The right femoral artery was cannulated with a 20-gauge catheter, which served as the arterial inflow port from the CPB circuit.

After surgical preparation, verification of the baseline blood gas values within normal range and activated clotting time (ACT) $> 300 \text{ s}$ (i-STAT, Heska, East Windsor, NJ, USA), the rats were paralyzed with intravenous vecuronium 2 mg/kg 5 min before asphyxia was begun. Animals were kept on the ventilator at a FiO_2 of 0.5 to maintain normal SpO_2 . After 5 min, asphyxiation was induced by disconnecting the animal from the ventilator. Cardiac arrest was defined to occur when mean arterial pressure (MAP) dropped below 20 mmHg. Duration of asphyxia was 8 min and body temperature was maintained at $37 \pm 0.5^\circ\text{C}$ during that period of time.

The customized CPB circuit designed for rodents consisted of a heat exchanger, an open venous reservoir, a membrane oxygenator (Martin Humbs, Ingenieurbüro für Feinwerktechnik, Valley, Germany), silicone tubing (i.d. 1.6 mm) and a roller pump (Masterflex, Barnant, Barrington, IL, USA) as previously described.^{13,14} The CPB circuit was primed with 12 ml Plasma-Lyte A (Baxter, Deerfield, IL, USA). As needed, additional

Plasma-Lyte A solution was added to the venous reservoir to maintain a constant circulating volume. After 8 min of asphyxia, resuscitation was started with the initiation of CPB flow. The CPB flow rates reached 150–160 ml/kg/min within 1 min, which approximates normal cardiac output in the rat.¹⁵

Temperature control during CPB was achieved using a circulating water bath heat exchanger which was integral within the oxygenator. A blended gas of 98% O₂/2% CO₂ was introduced into the oxygenator at a gas/CPB flow ratio of 2:1, thus providing oxygenation while preventing hypocapnia. ACT was maintained at >300 s during the CPB period. Arterial blood samples following 5, 15, 30, 45 and 60 min of CPB were obtained for blood gas analysis (α -stat). A base excess below –3 mmol/l was corrected by a 0.1 ml bolus dose of sodium bicarbonate (0.9 mmol/ml). Following ROSC, the CPB flow rate was decreased gradually to half the original flow rate after 40 min of CPB to prevent excessive volume administration. This flow rate was maintained for the final 20 min of CPB support. When a stable hemodynamic status defined as a MAP > 80 mmHg was achieved, mechanical ventilation was restarted with FiO₂ 0.5, PEEP 2 cm H₂O, TV 6 ml/kg and RR 25–30 min^{–1} to prevent atelectasis and ventilation–perfusion distribution mismatch. TV was regulated to maintain PaCO₂ between 35 and 45 mmHg. Isoflurane was delivered via the membrane oxygenator and the ventilator for maintenance of adequate depth of anesthesia as needed. No vasopressors were used during CPB resuscitation.

The 1 h of ICU-like care started with discontinuation of the CPB support but included ventilator support. After discontinuation of CPB, the remaining blood in the CPB circuit was collected and slowly reinfused into the animal. Heparinization was discontinued and allowed to metabolize to a normal coagulation profile without any medication. Mechanical ventilation with FiO₂ 1.0, PEEP 2 cm H₂O, TV 8–10 ml/kg and RR 40 min^{–1} were continued to maintain normocapnia. A 7 g wireless probe (TA10TA-F40, Data Sciences International, St. Paul, MN, USA) was implanted through a midline laparotomy incision into the peritoneal cavity of the rat for post-operative temperature control. Rats were extubated after the 1 h of intensive care, all catheters were removed, and incision sites were sutured. The rats were placed separately in special temperature-feedback control cubicles for precise maintenance of body temperature during the 6 h post-ventilation support period. Animals were given supplemental oxygen overnight and free access to food and water. Body weight was assessed daily. If rats lost 10% of the initial body weight, 10 ml Normosol-R/5% Dextrose injection was given subcutaneously.

Overall performance categories¹⁶ (OPC; 1 = normal, 2 = moderate disability, 3 = severe disability but conscious, 4 = coma, and 5 = death) and revised neurological deficit scores¹⁷ (NDS; 0–10% = normal, 100% = brain death) were evaluated at 24, 48 and 72 h post-resuscitation.

2.2. Experimental protocol

The experimental protocol was developed which (Figure 1) included six sequential phases. (1) Baseline allowed for a stable condition following all surgical procedures under isoflurane anesthesia. For 5 min prior to asphyxia, the isoflurane was washed out while the previously established ventilatory parameters were maintained. (2) Complete asphyxia for 8 min. (3)

ECPB resuscitation was initiated for 1 h and then gradually discontinued. (4) Intensive care and ventilatory support for 1 h. (5) Body temperature was strictly controlled for another 6 h. (6) Animals were observed for up to additional 3 days.

Immediately before ECPB resuscitation, the animals were block randomized into 3 groups ($n = 10$ in each group): normothermic animals (NORM) were maintained at 37 °C during ECPB, ventilatory support and temperature control phases; animals treated with mild hypothermia (MILD) were cooled and maintained at 34 °C during ECPB, ventilatory support and temperature control phases; animals treated with moderate hypothermia (MOD) were cooled to and maintained at 30 °C during the ECPB period, then rewarmed from 30 °C gradually up to 34 °C during the ventilatory support phase of 1 h, and were maintained at 34 °C during the temperature control phase of 6 h. In addition, sham animals ($n = 5$) received anesthesia, ventilation, and surgical procedures for brain histology controls. If hemorrhage occurred as a result of cannulation or other surgical procedures or if gasping occurred during the asphyxial period, the animals were considered a technical failure and eliminated from the primary analysis.

2.3. Histology

The brains of the animals of the three groups were histologically examined following termination of study and euthanasia. In addition, sham animals that received identical duration of anesthesia, ventilation and surgical procedures but had no cardiac arrest, CPB or temperature-feedback control phases, were also studied to compare with the other surviving animals. Immediately prior to euthanasia, all surviving animals (including sham animals) were re-anesthetized with isoflurane on the day 3 after the arrest/resuscitation. Animals were perfused via left ventricle with 100 ml of normal saline followed by 200 ml of 4% paraformaldehyde. Brains were immediately removed and placed in 4% paraformaldehyde for at least 24 h. Prior to sequential sectioning, brains were serially submersed in 10%, 20% and 30% sucrose in 0.1 mol/l PBS (pH 7.4) until sinking in each solution. Coronal sections (20 μ m) were cut sequentially on a freezing microtome. Five sections of each brain were selected at every 240 μ m starting from Bregma -2.3 mm. Sections were stained with 5 μ g/ml Hoechst 33342 (Sigma, St. Louis, MO, USA). Photomicrographs were taken using a Leica DM4500 B microscope (Leica Microsystems Ltd., Heerbrugg, Switzerland). Labeled surviving neurons per high-power field (400 $\times$) of hippocampus in medial, middle and lateral sectors of CA1 in both right and left hemispheres were counted using Adobe Photoshop CS3 (Adobe Systems Inc., San Jose, CA, USA).^{18,19} Neuronal cell counts were conducted by an observer who was blinded to the treatment group.

2.4. Statistical analysis

Data were presented as mean $\pm$ standard deviation unless otherwise stated. One-way ANOVA was performed, followed by Bonferroni post hoc tests to identify differences between groups in physiologic parameters, blood gas results and hippocampus CA1 cell counting results. A Chi-square test was used to test for differences in survival and proportions of OPC values (favorable versus unfavorable outcome, OPC 1–2 versus OPC 3–5) between groups. Kruskal–Wallis test and Mann–Whitney U test were used to compare NDS between groups. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Physiologic variables

Baseline measurements including animal weight, hematologic and biochemical parameters, acid–base status, temperature, and hemodynamic parameters did not vary between groups (Table 1). The duration from asphyxia to cardiac arrest was not different among NORM, MILD and MOD groups (216 ± 38 , 216 ± 14 and 205 ± 15 s, respectively). The rats in all experimental groups were successfully resuscitated by ECPB. There was no significant difference in the time from onset of CPB to ROSC among NORM, MILD, and MOD groups (47 ± 25 , 97 ± 103 and 43 ± 9 s, respectively). Temperature and blood pressure changed as expected according to the experimental protocol. Sodium bicarbonate administration, the amount of fluid administration, the heparin dosage and ACT were not different among groups. The blood gas values during the experiments are shown in Table 1. Hematocrit (Hct) was significantly decreased compared to baseline within each group after ECPB resuscitation, however there was no difference between these groups at each time point.

3.2. Survival and neurological outcome

Animal survival is shown in Figure 2. Three rats in the NORM group died immediately after discontinuation from assisted ventilation due to respiratory failure. Seizures were observed in two NORM animals when anesthesia was discontinued. The 72 h survival was 0% (0 of 10) in the NORM group, 60% (6 of 10) in the MILD group and 80% (8 of 10) in the MOD group. Survival within the hypothermia groups was significantly higher than the NORM group ($p < 0.05$).

At 24 h post-resuscitation, NDS was best in the MOD group compared to the other groups ($p < 0.05$, Figure 3), and there were no significantly difference between the MILD group and the NORM group. NDS were not significantly different between the MILD group and the MOD group at 48 and 72 h post-resuscitation. There are no NDS data in the NORM group at 48 and 72 h post-resuscitation for compare because all NORM animals dead before 48 h post-resuscitation could not be proved by brain death. In the MOD group, 7 of 10 animals achieved the favorable outcome (OPC 1 or 2) at 72 h post-resuscitation, compared to 3 of 10 in the MILD group and 0 of 10 in the NORM group (Figure 4). The OPC was significantly different in the MOD group compared to the NORM group ($p = 0.003$).

3.3. Visceral complications

In the NORM group, nine animals had pulmonary edema, three animals had small areas of liver necrosis, one animal had small areas of splenic necrosis, and two animals had hemoperitoneum due to liver injury (not attributable to the surgical procedures). In the MILD group, three animals were found to have pulmonary edema, two animals had small areas of liver and splenic necrosis, one animal had evidence of kidney necrosis, two animals had a hemoperitoneum with one animal showing liver injury. In the MOD group, three animals were found to have pulmonary edema, one animal had small patches of liver and kidney necrosis and one animal had a subcapsular renal hematoma. Anuria or hematuria observed in the experimental animals was closely associated with mortality.

3.4. Hippocampus CA1 histology and neuronal cell counting

Significant loss of neurons was observed in the MOD group when compared to sham ($p = 0.04$), but there was no statistical difference between the MILD group and sham nor between the MILD and MOD groups (Figure 5). There were no surviving NORM animals for a proper comparison as none survived to 72 h for exam.

4. Discussion

This is the first report that the authors could identify for the use of a rodent model of ECPB resuscitation in the setting of asphyxial cardiac arrest. Our data confirmed the important adjunctive role of early hypothermia in resuscitation using ECPB techniques following cardiac arrest. The addition of early hypothermia to ECPB resuscitation was associated with a benefit in survival from 0% to 80% in this preliminary study. The addition of both mild and moderate hypothermia to ECPB resuscitation appears to significantly improve the survival and neurological recovery in asphyxial cardiac arrest in rats as compared with normothermic ECPB resuscitation.

The use of mild and moderate hypothermia as an adjunct to ECPB resuscitation after cardiac arrest has been shown to improve cerebral outcome in dogs compared to normothermic ECPB, whereas deep post-arrest hypothermia can worsen cerebral and cardiac outcome.²⁰ Ao et al.²¹ compared long-term (20 h) mild hypothermic ECPB (33 °C) with normothermic ECPB (37.5 °C) after 15 min of untreated ventricular fibrillation in dogs. The results showed that ECPB with mild hypothermia induced immediately after cardiac arrest improved survival as well as cerebral and cardiac outcomes compared to normothermic ECPB. Our data on the neurological recovery are in line with these reports.

Our findings are consistent with recent human clinical data and experimental studies. Some clinical studies of ECPB resuscitation have used hypothermia as part of the resuscitation protocol; these studies suggest the application of hypothermia may be important. In an out-of-hospital cardiac arrest study, the rate of survival increased significantly with the use of ECPB with hypothermia compared to standard cardiopulmonary resuscitation (CPR, 22.7% vs. 9.9%).²² In another study, Nagao et al.⁷ reported on ECPB, also with hypothermia, in patients with out-of-hospital cardiac arrest who did not respond to standard ACLS. Eighty-nine percent of these patients achieved ROSC, and 25% of the patients survived. However, early hypothermia was not attempted in these studies. In an experimental study, Abella et al.²³ demonstrated that intra-arrest moderate hypothermia and “cool reperfusion” during resuscitation could significantly improve survival compared to delayed cooling 20 min after resuscitation. Despite the studies that suggest protection with hypothermia in the setting of ECPB, the issue is not settled and it should be noted that there are other studies which do not use hypothermia as part of a resuscitation protocol.^{24,25} Exploration of the full potential benefit and critical timing for therapeutic hypothermia in the setting of ECPB should be the subject of an additional study.

A surprising finding in this study included some cases of liver injury, small areas of necrosis in various organs, and a subcapsular renal hematoma in experimental animals. In the early stages of this study, we excluded these animals as “out of protocol due to the surgical error.”

But with the frequency of these same complications, and after a careful review of the surgical procedures that provided no reason for this kind of injury, we reasoned that these complications were consequences of the CPB technique or anticoagulation but not due to surgical failure and we included these animals in the analysis. Future investigators using ECPB should be vigilant for these complications.

The rat asphyxial cardiac arrest model we used in this study is well described and widely accepted and has been employed in numerous cardiac arrest studies.^{26–28} There are also ventricular fibrillation (VF) models in rats in which VF is induced by alternating current delivered to the right ventricular endocardium.^{29,30} But this procedure is somewhat complicated for its invasive method, its possible interference with cerebral hemodynamic by placing an additional jugular catheter, its potential to produce thermal injury of the endocardium at the site of the electrode, and the long yet variable duration of AC application required for VF induction and maintenance.^{29–33} These disadvantages limit the validity of this technique as a correlate to naturally occurring human VF arrest.

There were important limitations in our study. The survival of the rats in our study with normothermic CPB resuscitation was lower than the survival with normothermic standard ACLS CPR in some previous reports.^{26–28} This may be due to the fact that the model of ECPB resuscitation in this study is not designed to exploit all possibilities to achieve optimal survival rates and comparing those to standard ACLS CPR, but rather to test the utility of hypothermia during early reperfusion as an adjunct to a basic model of ECPB resuscitation. Numerous other interventions have the potential to improve the survival benefit in conjunction with ECPB such as the pharmacological, immunological and rheological modification of the perfusate, addition of vasopressors, blood transfusions for anemia, longer periods of cooling, and more prolonged ICU care. These need to be explored in future studies. Moreover, the survival rates found in this study many also indicate that ECPB by its invasive nature could be inferior to standard CPR if the arrest duration is relatively short, and that a certain extent of injury could be required to allow it to become superior.

As noted, all animals developed anemia (lowest Hct about 23%) after ECPB resuscitation and this would likely not be seen in human ECPB procedures. We did not transfuse our animals with donor blood and no anti-heparinization drugs were used after ECPB resuscitation in this experiment. While this did not vary by group, the anemia was not an intentional part of the model. Data from a normothermic CPB experimental study in dogs demonstrated that a Hct of 25% was sufficient to match whole-body oxygen consumption, but a Hct < 18% was unsafe.³⁴ The hypothermia protocol that we used was relatively short compared with current clinical practice because experimental animals appeared to become dehydrated by our temperature control methods (cooling fan and heating lamp) if longer temperature control was performed. This is relevant because post-resuscitation hypothermia in most human studies was provided over 12 h or even longer,^{35–38} whereas cooling in the present study was maintained for only 8 h. We do not believe that an optimal hypothermia strategy for use with ECPB has yet been developed or tested, so we may see additional benefit with longer cooling periods. Finally, our histopathology results are not able to provide data on hypothermic versus normothermic animals because all of the normothermic animals died prior to 72 h.

5. Conclusions

We have developed a basic rat model of ECPB resuscitation in asphyxial cardiac arrest that may prove useful in future studies. The addition of early mild and moderate hypothermia to ECPB resuscitation significantly improved survival compared with normothermic ECPB. Clinicians using ECPB should be aware that hypothermia appears to be an important adjunct to the use of ECPB resuscitation although many questions remain unanswered for its optimal use.

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Figure 1.

Experimental protocol. T_{ty}, Tympanic temperature; CPB, cardiopulmonary bypass; ICU, intensive care; Temp, temperature; RT, resuscitation time.

Figure 2. Survival after emergency cardiopulmonary bypass resuscitation in different groups. * $p < 0.05$, compared to the NORM group.

Figure 3.

Neurological deficit scores (NDS) at 24, 48 and 72 h post-resuscitation. Boxes represent interquartile ranges. The line across each box indicates the median, and the whiskers are the highest and lowest values. Post-resuscitation 24 h: $p < 0.05$, the MOD group compared to the other groups; there were no significantly difference between the NORM group and the MILD group. Post-resuscitation 48 and 72 h: there were no significantly difference between the MILD group and the MOD group. There are no NDS data in the NORM group at 48 and 72 h post-resuscitation for compare because all NORM animals dead before 48 h post-resuscitation could not be proved by brain death.

		NORM	MILD	MOD
Unfavorable	OPC 5 (Death)	●●●●● ●●●●●	●●●●	●●
	OPC 4 (Coma)			
	OPC 3 (Severe disability)		●●●	●
Favorable	OPC 2 (Moderate disability)			●●
	OPC 1 (Normal)		●●●	●●●●●

Figure 4.

Overall performance categories (OPC, favorable vs. unfavorable outcome, OPC 1–2 vs. OPC 3–5) at 72 h post-resuscitation. $p = 0.003$, the MOD group compared to the NORM group. Each dot represents one rat.

Figure 5.

The neuronal survival in hippocampus CA1 at 72 h post-resuscitation. (A) The representative field of medial sectors of hippocampus CA1 neurons in sham animals ($n = 6$). (B and C) The representative fields of damaged medial sectors of hippocampus CA1 neurons in the MILD group ($n = 6$) and the MOD group ($n = 8$); each marker in (D) represents the mean number of surviving neurons per field for a single rat. $*p = 0.04$, the MOD group compared to sham. Scale bar, 50 μm ; magnification, 400 $\times$.

Table 1

Blood gas parameters during experiments.

	Baseline	CPB 60min	ICU 60min	Final
pHa				
NORM	7.43 ± 0.04	7.39 ± 0.13	7.34 ± 0.15	NA
MILD	7.42 ± 0.03	7.43 ± 0.09	7.45 ± 0.06	7.53 ± 0.08
MOD	7.43 ± 0.03	7.44 ± 0.03	7.43 ± 0.03	7.52 ± 0.05
PaO ₂ (mmHg)				
NORM	280 ± 26	343 ± 112	435 ± 81	NA
MILD	278 ± 91	430 ± 72	442 ± 96	427 ± 95
MOD	277 ± 50	500 ± 70 [*]	491 ± 55	414 ± 132
PaCO ₂ (mmHg)				
NORM	39.2 ± 3.7	52.0 ± 20.9	50.3 ± 9.7	NA
MILD	40.4 ± 4.5	49.0 ± 17.1	44.6 ± 5.4	33.4 ± 6.2
MOD	39.4 ± 2.5	42.6 ± 2.8	47.5 ± 3.5	36.6 ± 4.5
Base excess (mmol/l)				
NORM	2.1 ± 1.5	4.8 ± 2.9	1.5 ± 7.1	NA
MILD	1.9 ± 1.4	5.1 ± 1.7	7.0 ± 2.5 [*]	4.5 ± 3.1
MOD	1.7 ± 1.5	4.7 ± 1.6	7.4 ± 1.8 [*]	6.4 ± 1.7
Hematocrit (%)				
NORM	37.0 ± 2.4	25.0 ± 2.5	30.0 ± 5.1	NA
MILD	35.4 ± 2.3	24.4 ± 3.1	27.8 ± 2.4	23.7 ± 2.8
MOD	37.2 ± 2.0	27.2 ± 1.9	31.0 ± 2.0	23.1 ± 4.7
Glucose (mg/dl)				
NORM	288 ± 81	120 ± 12	115 ± 24	NA
MILD	254 ± 64	166 ± 64	167 ± 20 [†]	237 ± 19
MOD	273 ± 57	259 ± 44 [†]	133 ± 32	240 ± 25

CPB, Cardiopulmonary bypass; ICU, intensive care; NA indicates not applicable.

^{*} $p < 0.05$, compared to the NORM group at the same time point.[†] $p < 0.05$, compared to the other groups at the same time point.