ORIGINAL RESEARCH

Hyperoxygenation With Cardiopulmonary Resuscitation and Targeted Temperature Management Improves Post–Cardiac Arrest Outcomes in Rats

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BACKGROUND: Oxygen plays a pivotal role in cardiopulmonary resuscitation (CPR) and postresuscitation intervention for cardiac arrest. However, the optimal method to reoxygenate patients has not been determined. This study investigated the effect of timing of hyperoxygenation on neurological outcomes in cardiac arrest/CPR rats treated with targeted temperature management.

METHODS AND RESULTS: After induction of ventricular fibrillation, male Sprague-Dawley rats were randomized into 4 groups (n=16/group): (1) normoxic control; (2) O_2_CPR, ventilated with 100% O_2 during CPR; (3) O_2_CPR+postresuscitation, ventilated with 100% O_2 during CPR and the first 3 hours of postresuscitation; and (4) O_2_postresuscitation, ventilated with 100% O_2 during the first 3 hours of postresuscitation. Targeted temperature management was induced immediately after resuscitation and maintained for 3 hours in all animals. Postresuscitation hemodynamics, neurological recovery, and pathological analysis were assessed. Brain tissues of additional rats undergoing the same experimental procedure were harvested for ELISA-based quantification assays of oxidative stress–related biomarkers and compared with the sham-operated rats (n=6/group). We found that postresuscitation mean arterial pressure and quantitative electroencephalogram activity were significantly increased, whereas astroglial protein S100B, degenerated neurons, oxidative stress–related biomarkers, and neurologic deficit scores were significantly reduced in the O_2_CPR+postresuscitation group compared with the normoxic control group. In addition, 96-hour survival rates were significantly improved in all of the hyperoxygenation groups.

CONCLUSIONS: In this cardiac arrest/CPR rat model, hyperoxygenation coupled with targeted temperature management attenuates ischemia/reperfusion-induced injuries and improves survival rates. The beneficial effects of high-concentration oxygen are timing and duration dependent. Hyperoxygenation commenced with CPR, which improves outcomes when administered during hypothermia.

Key Words: cardiac arrest ■ cardiopulmonary resuscitation ■ hyperoxygenation ■ neurological outcome ■ oxidative stress ■ targeted temperature management

Cardiac arrest (CA) leads to the immediate cessation of blood flow and oxygen delivery.[1] Hypoxia results in a series of cellular damage and may induce permanent myocardial and neuronal injuries.[2] The goal of cardiopulmonary resuscitation (CPR) is to restore spontaneous circulation and to prevent hypoxic ischemic brain injury.[3] Given that adequate oxygen delivery is necessary to restore the energy state of the heart and to maintain the energy state of the brain to minimize ischemic injury,[4] the guidelines for adult CPR

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CLINICAL PERSPECTIVE

What Is New?

- This study finds that hyperoxygenation administered during cardiopulmonary resuscitation and postresuscitation targeted temperature management significantly improves neurological outcome in adult rats.
- The improved hemodynamic stability and myocardial function together with attenuated brain injury and improved cerebral recovery resulted in better outcome when hyperoxygenation is coupled with targeted temperature management.
- Hyperoxygenation and targeted temperature management synergistically exert protective effects by providing adequate oxygen delivery and neutralizing the excessive reactive oxygen species.

What Are the Clinical Implications?

- The beneficial effects of post–cardiac arrest high-concentration oxygen ventilation are timing and duration dependent because hyperoxygenation can reduce the hypoxia-related tissue damage but may simultaneously increase the risk of excessive production of reactive oxygen species.
- High-concentration oxygen coupled with targeted temperature management may be applicable to persistently comatose adults experiencing ventricular fibrillation cardiac arrest and cardiopulmonary resuscitation.

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Nonstandard Abbreviations and Acronyms

CA	cardiac arrest
NC	normoxic control
NDS	neurological deficit score
ROS	reactive oxygen species
TTM	targeted temperature management

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and emergency cardiovascular care recommend the use of maximal feasible inspired oxygen concentrations during CPR and earlier postresuscitation.[5] The rationale for high-concentration oxygen use is the belief that hyperoxic ventilation should increase oxygen delivery to the ischemic tissues, thereby reducing hypoxia-related mortality and morbidity.[6]

In recent years, a considerable amount of data has emerged challenging the appropriateness of the use of 100% O_2 during and after resuscitation from CA.[7] Animal studies unanimously reported that hyperoxic ventilation was associated with more adverse neurological outcomes compared with lower

oxygen concentrations in different models of CA.[3,8] Hyperoxia has been suggested to increase the generation of reactive oxygen species (ROS), resulting in increased reperfusion neuronal injury and worsened neurological outcomes.[9] Few studies to date, however, have correlated long-term neurological injuries with biochemical evidence of oxidative stress. Clinical studies examining the influence of hyperoxia on outcomes in patients resuscitated from CA have provided conflicting results.[10–12] Unfortunately, previous studies have focused mainly on oxygenation during postresuscitation care. In addition, given the discrepancies and confounders in arrest locations, variable delays to oxygen management, and application of targeted temperature management (TTM) in clinical scenarios, it remains unclear whether the effect of hyperoxic ventilation is timing and/or dose dependent, and the optimal method to reoxygenate patients with CA remains unknown.[13]

The present study was designed to investigate the effects of different timing of 100% O_2 ventilation during CPR and early postresuscitation TTM on neurological outcomes and oxidative stress in a CA/CPR rat model. We hypothesized that hyperoxygenation coupled with TTM would improve post-CA outcomes.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement

Ethical approval for this animal study was granted by Laboratory Animal Welfare and Ethics Committee of the Army Medical University (SGX2018yjs02).

Experimental Animals

A total of 100 healthy, male Sprague-Dawley rats weighing between 310 and 410 g supplied by the laboratory animal center of the Army Medical University were used in the study. We used male rats because the majority of patients with CA are male. Among these rats, 88 rats were used to establish the CA/CPR model, and the remaining 12 rats did not experience CA and CPR and were assigned to a sham-operated group (6 for pathological analysis and 6 for assessment of oxidative stress).[14] In the CA/CPR model, 64 rats were used for the measurement of hemodynamics, electrophysiology, blood gas, biochemical markers of cerebral injury, 96-hour neurological outcomes, and pathological analysis, and the remaining 24 rats were used for ELISA-based quantification assays of oxidative stress-related molecular biomarkers 6 hours after resuscitation.

Animal Preparation

All animals were housed under controlled laboratory conditions (temperature $22.0\pm2.0°C$; 12-hour normal dark–light cycle) with free access to chow and water. Before the procedure, the animals were fasted overnight except for free access to water. Anesthesia was initiated with intraperitoneal injection of pentobarbital sodium (45 mg/kg), and additional doses (10 mg/kg) were administered at ≈1-hour intervals or when required to maintain anesthesia. Rats were fixed in supine position on a surgical board for the operative procedure. Three subcutaneous needle electrodes were inserted into the limbs for ECG measurement. Four subdermal needles were inserted into the surfaces of the skull for electroencephalogram measurement. After endotracheal intubation with a 14-gauge cannula, the animals were mechanically ventilated through a volume-controlled ventilator with a tidal volume of 0.65 mL/100 g at a FiO_2 of 0.21 (ALC-V8, Alcott Biotech Co. Ltd, Shanghai, China). The right femoral artery was cannulated with a PE-50 catheter for invasive blood pressure monitoring and arterial blood samples. An additional PE-50 catheter was advanced into the left femoral vein for fluid and drug administration. All of the catheters were flushed intermittently with saline solution containing 2.5 IU/mL of heparin. With the aid of an esophageal probe, body temperature was continuously monitored and maintained at $37.0\pm0.5°C$ with an overhead heating lamp in the preparation phase.

Experimental Procedures and Randomization

The experimental protocol is shown in Figure 1. After baseline measurement, mechanical ventilation was ceased, and VF was induced by 5 mA/50 Hz transesophageal alternating current stimulation. The stimulation was applied for 2 minutes to prevent spontaneous defibrillation. The presence of CA was confirmed by an abrupt decrease in mean arterial pressure (MAP) below 20 mm Hg and irregular deflections of varying amplitude and contour in the ECG waveform.

After the successful induction of CA, animals were randomized into 4 groups using a randomized number sequence (n=16/group): (1) normoxic control (NC); (2) O$_2$_CPR, ventilated with 100% O$_2$ during CPR; (3) O$_2$_CPR+postresuscitation, ventilated with 100% O$_2$ during CPR and the first 3 hours of postresuscitation; and (4) O$_2$_postresuscitation, ventilated with 100% O$_2$ during the first 3 hours of postresuscitation. Animals were ventilated with 21% O$_2$ during the remainder of the experimental time.

After 7 minutes of untreated CA, mechanical ventilation was initiated with either 21% O$_2$ or 100% O$_2$ according to the randomization. Manual external chest compression was performed at a rate of 240 compressions/min with equal compression–relaxation duration, and the depth of compressions was initially adjusted to maintain a MAP >20 mm Hg. A 0.04 mg/kg bolus of epinephrine was injected 1 minute after the start of CPR. After 3 minutes of CPR, resuscitation was attempted with single 2 J defibrillations (M-Series, Zoll Medical Corporation, Chelmsford, MA). If sinus rhythm was not achieved, an additional dose of epinephrine was administered, and resuscitation continued with defibrillation every 30 seconds. CPR was continued until the return of spontaneous circulation (ROSC) or repeated for a maximum of 3 cycles. Successful resuscitation was defined as a supraventricular rhythm with MAP >60 mm Hg lasting for at least 5 minutes.

After resuscitation, the animals received intensive care for 6 hours. The body temperature was reduced to 34.0°C using ice packs and an electrical fan.[7,14] TTM was used in all animals because it is strongly recommended by the guidelines as a standard component of postresuscitation care for patients with ROSC from ventricular fibrillation.[15] The cooling duration of the maintenance phase of 34.0°C was the first 2 hours of postresuscitation. Rewarming was achieved by gradually increasing the temperature until the rats reached 37.0°C within 2 hours. All catheters, including the endotracheal tube, were removed, and incisions were closed 6 hours after resuscitation.

Postresuscitation Hemodynamics and Electrophysiology

Arterial pressure, ECG, and electroencephalogram waveforms were constantly measured and recorded for 6 hours via a personal computer–based data acquisition system supported by WINDAQ software (DATAQ Instruments Inc., Akron, OH). Weighted-permutation entropy, a characteristic of the electroencephalogram for the early prediction of neurological outcome, was quantitatively analyzed.[16] LVEF, an indicator of myocardial contractility, was noninvasively assessed at baseline and at hourly intervals after resuscitation using an echocardiograph system (DC-6, Mindray Medical International Limited, Shenzhen, China).

Blood Gas and Biochemical Markers of Cerebral and Cardiac Injuries

Arterial blood samples were drawn from the right femoral artery at baseline, 5 minutes, 3 hours, and 6 hours after resuscitation. Blood gases were measured with the aid of a blood analyzer (i-STAT, Abbott Point of Care Inc, Abbott Park, IL) for each blood sample. The serum concentration of astroglial protein S100B and cardiac troponin T were quantified with an enzyme-linked immunoassay (Elisa Kit, Cusbio
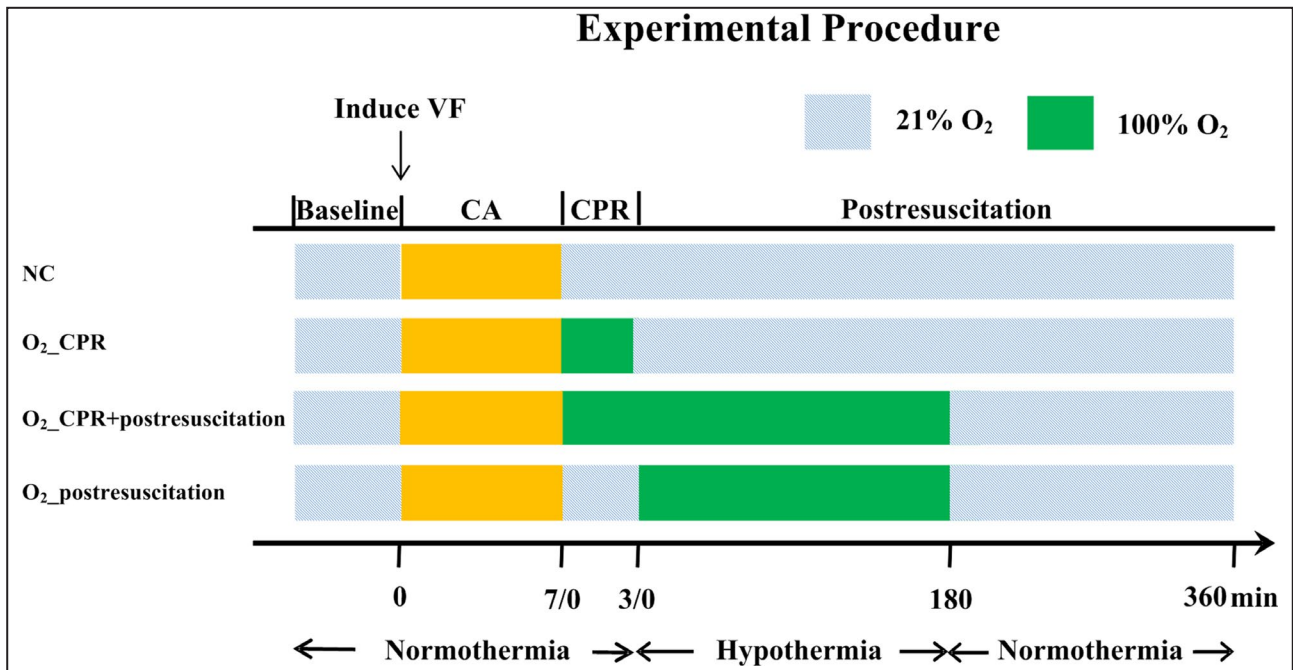
Figure 1. Experimental protocol and different timing of hyperoxygenation in each group (n=16/group).
CA indicates cardiac arrest; CPR, cardiopulmonary resuscitation; NC, normoxic control; O_2_CPR, ventilated with 100% O_2 during CPR; O_2_CPR+postresuscitation, ventilated with 100% O_2 during CPR and the first 3 hours of postresuscitation; O_2_postresuscitation, ventilated with 100% O_2 during the first 3 hours of postresuscitation; and VF, ventricular fibrillation.

Biotech Co. Ltd., Wuhan, China) according to the manufacturer's instructions at baseline and 3 hours and 6 hours after resuscitation. The calcium binding protein S100B is a biomarker that predicts the progress or the prognosis of brain injury after CA.[17] The cardiac-specific contractile protein cardiac troponin T is a biochemical marker that allows the detection of myocardial damage because it is released into the blood only after myocardial injury.[18]

Neurologic Evaluation and Survival Observation

The neurological deficit score (NDS) and adverse events were examined at 24, 48, 72, and 96 hours after ROSC and confirmed by 2 observers blinded to prior treatments. Consciousness and breathing frequency, cornea and cranial nerve reflexes, motor sensory function, auditory reflex, and coordination were scored according to a normative NDS system (0–500 scale: 0, no observed neurological deficit; 500, without any brain function), and the average score of the 2 observers was used to evaluate the neurological appearance after global cerebral ischemia.[19] The 96-hour survival times were recorded for cumulative overall survival analysis.

Pathological Examination

After 96 hours of evaluation of the final NDS, the surviving animals and 6 sham-operated rats were anesthetized with sodium pentobarbital. The brains were removed and immersion fixed in paraformaldehyde. After embedded in paraffin, the brains were sliced in 5-µm sections on a microtome. The sections were stained with hematoxylin and eosin (Servicebio Technology Co, Ltd, Wuhan, China) for morphological evaluation, TUNEL (terminal deoxynucleotidal transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling) (Roche Applied Science, Basel, Switzerland) for apoptosis detection, and 4′,6-diamidino-2-phenylindole (DAPI) and fluorojade B (FJB) (Servicebio Technology Co, Ltd, Wuhan, China) to evaluate hippocampal neuronal cell degeneration.[20] The pyramidal cell layer in the CA1 region of the hippocampus was visually assessed under 3 nonoverlapping fields at ×400 magnification by 2 investigators blinded to the treatment assignment. For hematoxylin and eosin staining, morphological pattern, structure, and arrangement of neurons together with chromatin and nuclei were examined.[21] For TUNEL staining, neurons with brown staining, cell shrinkage, and nucleus condensation were examined.[22] For FJB staining, the pyramidal cell layer of CA1 was first localized with ultraviolet light (340–380 nm) using ×40 magnification. DAPI-positive neurons and FJB-positive neurons were then examined with ultraviolet light (340–380 nm) and blue excitation light (465–495 nm) using ×400 magnification.[23] Three fields of each DAPI/FJB sections were examined

using a defined rectangular field area (0.04 mm^2). The proportion of FJB-positive area and the number of FJB-positive and DAPI-positive neurons were computer-assisted semiquantitative analyzed with ImageJ software (version 1.46r, National Institutes of Health, Bethesda, MD). The ratio of FJB-positive neurons to the DAPI-positive neurons was used to evaluate neurodegeneration.

Oxidative Stress Biomarker Determination of Brain Tissue

The hippocampus and cortex of another 24 rats that underwent the same experimental procedure were removed 6 hours after ROSC. The organs were histologically compared with those of the 6 sham-operated rats. The brain tissue homogenate was centrifuged in 851g at 4°C for 10 minutes, and the supernatant was collected. The tissue protein concentration was quantified using a bicinchoninic acid protein assay kit (Nanjing JianCheng Technology Co, Ltd, Nanjing, China). The density of superoxide dismutase (SOD), malondialdehyde (MDA), and 8-hydroxy-2-deoxy guanosine (8-OHDG) were measured by commercial kits (Nanjing JianCheng Technology Co, Ltd, Nanjing, China). SOD is an antioxidant enzyme that reflects the ability to scavenge free radicals. MDA is the product of lipid peroxidation that exhibits a toxic effect in cells.[24] In addition, 8-OHDG is a widely used biomarker of oxidative damage to DNA.[25,26]

Statistical Analysis

A sample size of 16 animals in each group provided >80% power to detect a 30% increase in survival rate between groups with a type I error rate of 5%. Agreement of NDS between the 2 investigators was measured with κ statistics. The normal distribution of the data was confirmed using the Kolmogorov–Smirnov test. Normally and abnormally distributed data were reported as the mean±SD and medians including IQRs. Normally distributed variables of a single measurement were evaluated with ANOVA followed by post hoc Bonferroni correction. Normally distributed variables of multiple measurements were examined with the mixed effect model to determine the interaction of treatment group and time. If the interaction was significant, the simple main effect test was performed; otherwise, the main effect test was tested. ANOVA with Bonferroni correction for post hoc comparisons was used to show individual differences. Abnormally distributed variables were evaluated using the Kruskal–Wallis test followed by the Mann–Whitney U test for 2-group comparisons. Kaplan–Meier analysis and log-rank tests were used

for survival analysis. A $P<0.05$ was considered statistically significant. Statistical analyses were performed using R statistical software (version 3.1.2, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline Measurements and CPR Characteristics

No significant differences in baseline physiological measurements and resuscitation data were noted among the 4 groups (Table 1). All animals were successfully resuscitated and survived the 6-hour postresuscitation monitoring period with the exception of 3 animals in the NC group that died at 1.5, 5, and 5.3 hours. No exclusion or adverse events were observed during the experimental periods.

Postresuscitation Hemodynamics and Electrophysiology

The esophageal temperature and hemodynamic data before and after CA are shown in Figure 2. No significant differences in temperature and heart rate were noted among the groups (Figure 2A and 2B). Compared with the NC group, significantly increased MAP and LVEF were observed in the O$_2$_CPR+postresuscitation group within the first 5 hours postresuscitation (Figure 2C and 2D). However, no significant differences in MAP and LVEF were noted among the 3 groups with hyperoxygenation.

Electroencephalographic weighted-permutation entropy in the O$_2$_CPR+postresuscitation group was significantly increased in the NC group from 1 to 6 hours after ROSC (Figure 3A). However, no significant differences in weighted-permutation entropy were noted among the 3 groups ventilated with 100% O$_2$.

Blood Gas and Postresuscitation Cerebral and Cardiac Injuries

Table 2 presents arterial blood gas measurements. SaO$_2$, PaO$_2$, base excess, and total carbon dioxide capacity were significantly increased in the O$_2$_CPR and O$_2$_CPR+postresuscitation groups compared with the NC and O$_2$_postresuscitation groups 5 minutes after resuscitation. SaO$_2$, PaO$_2$, and total carbon dioxide capacity values measured at 180 minutes after resuscitation in the O$_2$_CPR+postresuscitation and O$_2$_postresuscitation groups were considerably increased compared with the NC and O$_2$_CPR groups. Lactate was markedly reduced in the O$_2$_CPR+postresuscitation group

Table 1. Baseline Variables and Characteristics of CPR

Variable	NC	O$_2$_CPR	O$_2$_CPR+Postresuscitation	O$_2$_Postresuscitation
Body weight, g	352.1±15.6	353.3±29.5	352.6±19.7	353.0±20.1
Heart rate, bpm	400.2±41.2	401.8±30.5	389.4±18.8	391.5±34.8
Temperature, °C	37.4±0.3	37.4±0.2	37.4±0.3	37.4±0.3
MAP, mm Hg	122.8±7.5	121.1±13.4	119.7±9.4	118.5±9.3
LVEF, %	83.6±4.3	83.4±2.9	82.9±4.6	82.9±5.7
CPR duration, s	217.8±30.3	213.7±27.7	208.8±29.3	212.8±24.0
Number of shocks, n	1.6±1.0	1.7±0.7	1.6±1.1	1.4±0.6
Total epinephrine, µg	22.3±10.2	19.5±7.6	17.3±6.4	18.2±6.2
ROSC rate, %	100	100	100	100

Data are shown as mean±SD. n=16/group. CPR indicates cardiopulmonary resuscitation; MAP, mean arterial pressure; NC, normoxic control; O$_2$_CPR, ventilated with 100% O$_2$ during CPR; O$_2$_CPR+postresuscitation, ventilated with 100% O$_2$ during CPR and the first 3 hours of postresuscitation; O$_2$_postresuscitation, ventilated with 100% O$_2$ during the first 3 hours of postresuscitation; and ROSC, return of spontaneous circulation.

compared with the NC group at 360 minutes after ROSC.

No differences in S100B and cardiac troponin T were noted at baseline (Figure 3B and 3C). Compared with the NC group, serum S100B levels measured at 180 and 360 minutes were significantly reduced in the O$_2$_CPR and O$_2$_CPR+postresuscitation groups (Figure 3B). In addition, serum cardiac troponin T
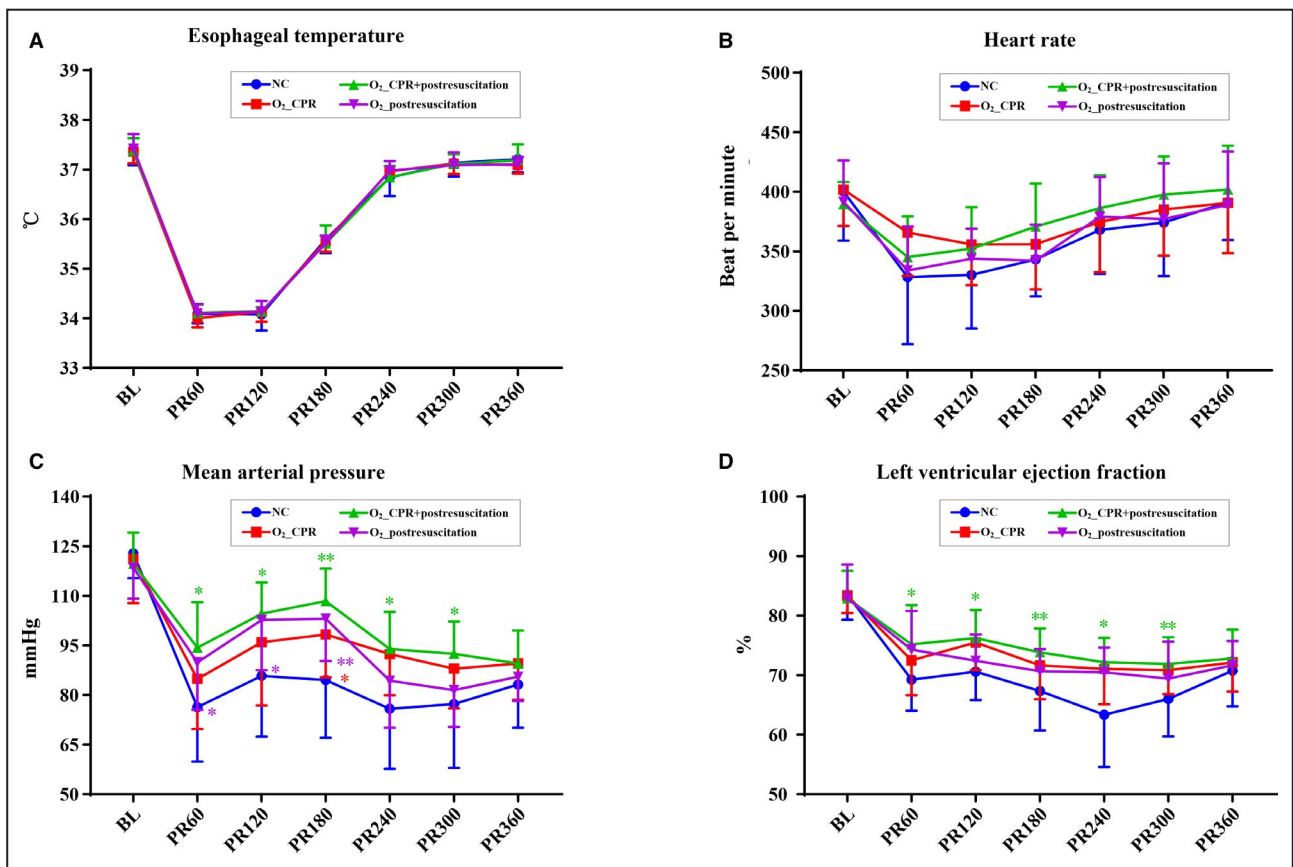

Figure 2. Esophageal temperature and hemodynamic data.
Esophageal temperature (**A**), heart rate (**B**), mean arterial pressure (**C**), and left ventricular ejection fraction (**D**) were measured at baseline (BL) and postresuscitation. NC indicates normoxic control; O$_2$_CPR, ventilated with 100% O$_2$ during CPR; O$_2$_CPR+postresuscitation, ventilated with 100% O$_2$ during CPR and the first 3 hours of postresuscitation; and O$_2$_postresuscitation, ventilated with 100% O$_2$ during the first 3 hours of postresuscitation; PR60, PR120, PR180, PR240, PR300 and PR360: 60, 120, 180, 240, 300 and 360 min postresuscitation. *P<0.05 compared with NC. **P<0.01 compared with NC. n=16 at each time point except for n=15 at PR120, PR180, and PR240; n=14 at PR300; and n=13 at PR360 in the NC group.
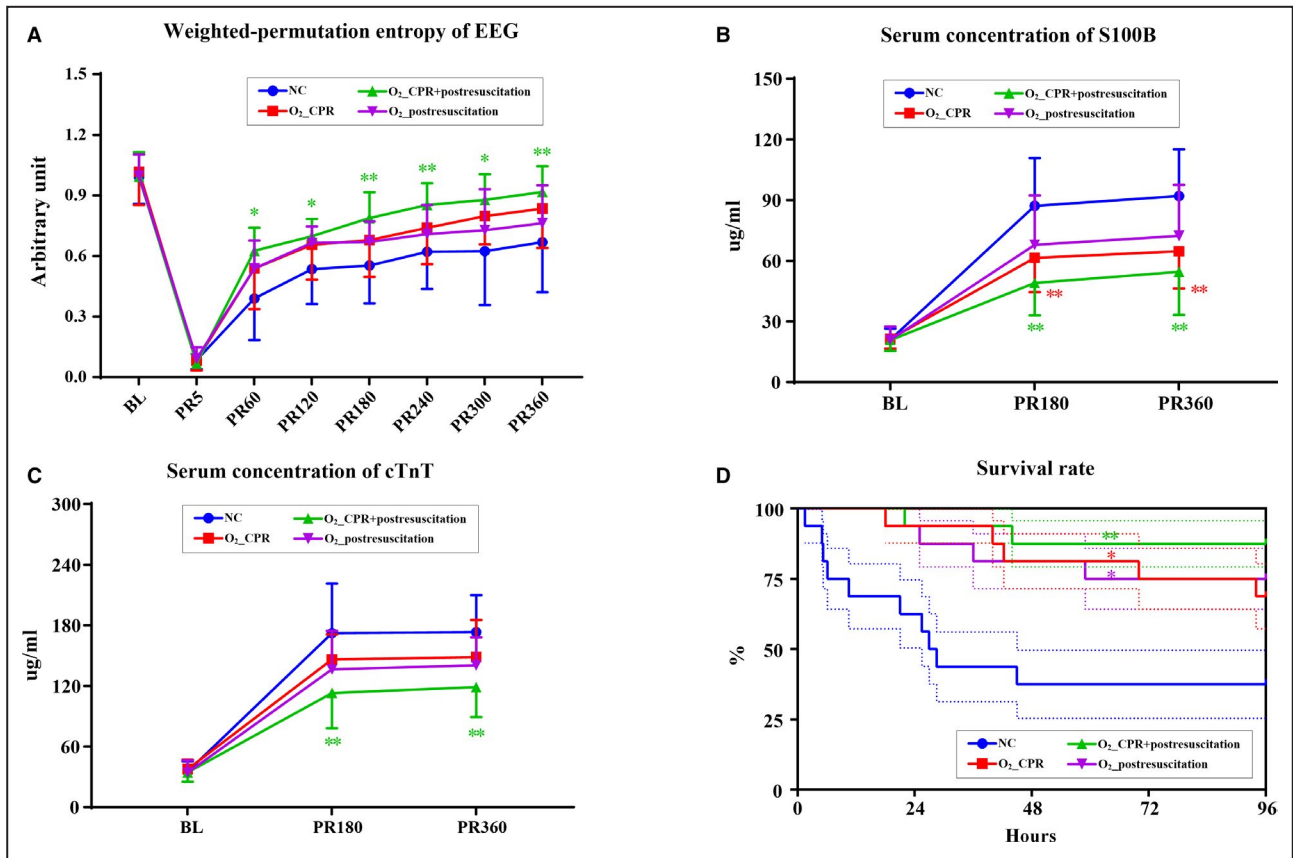
Figure 3. EEG weighted-permutation entropy and cerebral and cardiac injury and survival (n=16/group).
EEG weighted-permutation entropy (**A**), circulating levels of protein S100B (**B**), and cardiac troponin T (cTnT) (**C**) were measured at baseline (BL) and postresuscitation. Kaplan–Meier survival curve (**D**) was used for the cumulative overall survival analysis during the 96-hour observation period. CPR indicates cardiopulmonary resuscitation; EEG, electroencephalogram; NC, normoxic control; O_2_CPR, ventilated with 100% O_2 during CPR; O_2_CPR+postresuscitation, ventilated with 100% O_2 during CPR and the first 3 hours of postresuscitation; and O_2_postresuscitation, ventilated with 100% O_2 during the first 3 hours of postresuscitation; PR5, PR60, PR120, PR180, PR240, PR300 and PR360: 5, 60, 120, 180, 240, 300 and 360 min postresuscitation. *$P<0.05$ compared with NC; ** $P<0.01$ compared with NC. n=16 at each time point except for n=15 at PR120, PR180, and PR240; n=14 at PR300; and n=13 at PR360 in the NC group.

measurements after resuscitation were significantly reduced in the O_2_CPR+postresuscitation group compared with the NC group (Figure 3C).

Neurological Outcomes

NDS values measured during the 4 days following resuscitation are provided in Table 3. Statistical analysis showed strong consistency (κ value, 0.868) between the NDS values of the 2 observers. NDS values were significantly reduced in the O_2_CPR+postresuscitation group compared with the NC group. Six animals in the NC group survived to 96 hours compared with 11, 14, and 12 animals in the O_2_CPR, O_2_CPR+postresuscitation, and O_2_postresuscitation groups, respectively. As shown in Figure 3D, the cumulative 96-hour survival rate was significantly increased in the O_2_CPR (69% versus 38%; $P=0.03$), O_2_CPR+postresuscitation (88% versus 38%; $P=0.003$), and O_2_postresuscitation (75%

versus 38%; $P=0.018$) groups compared with the NC group. No significant differences in survival rates were noted among the 3 hyperoxygenation groups.

Pathological Analysis

Representative photomicrographs of the hematoxylin and eosin–stained CA1 region of the hippocampus from different animals in each group are presented in Figure 4. The modality configuration of cells was normal with intact structure and distinct nucleoli in the sham group. However, most neurons in the NC group exhibited irregular, polygonal, and spindle shapes with pyknosis, karyorrhexis, and karyolysis. The number of abnormal neurons was obviously decreased in the 3 groups treated with hyperoxygenation compared with the NC group.

Figure 5 shows cell apoptosis detected using the TUNEL method in the hippocampus CA1 sector from different animals in each group. Apoptotic cells were

Table 2. Arterial Blood Gas Analyses Before and After Resuscitation

Group	Baseline	PR5	PR180	PR360
SaO$_2$, %				
NC	94.8±1.5	87.6±3.6	97.2±1.5	94.8±4.2
O$_2$_CPR	93.4±2.3	97.6±2.0[†]	95.6±1.7	94.8±3.6
O$_2$_CPR+postresuscitation	94.9±2.5	98.4±2.3[†]	99.6±0.7[†,§]	96.6±1.8
O$_2$_postresuscitation	93.8±2.6	87.8±4.3[§,¶]	99.8±0.8[†,§]	95.3±2.5
PaO$_2$, mm Hg				
NC	74.4±6.0	77.4±15.3	88.1±8.7	82.1±14.3
O$_2$_CPR	69.3±9.5	196.5±67.4[†]	78.6±9.0	86.5±12.9
O$_2$_CPR+postresuscitation	75.9±8.0	186.5±70.1[†]	257.9±95.2[†,§]	88.3±11.5
O$_2$_postresuscitation	70.9±11.0	80.5±12.6[§,¶]	325.9±95.8[†,§]	78.9±13.5
PaCO$_2$, mm Hg				
NC	39.7±6.0	33.2±9.6	28.7±5.0	30.4±6.6
O$_2$_CPR	42.7±6.2	43.6±11.6	31.8±3.9	31.0±3.9
O$_2$_CPR+postresuscitation	41.1±5.3	44.0±11.4	37.1±6.0[†,‡,*]	33.3±3.5
O$_2$_postresuscitation	43.3±6.3	32.5±12.3[‡,‖,*]	33.0±5.2	33.0±4.9
Lactate, mmol/L				
NC	0.4±0.1	13.0±2.5	1.1±0.6	0.97±0.4
O$_2$_CPR	0.4±0.1	11.9±2.4	0.7±0.3	0.7±0.2
O$_2$_CPR+postresuscitation	0.4±0.1	12.1±2.0	0.5±0.2*	0.6±0.3*
O$_2$_postresuscitation	0.4±0.1	13.0±3.0	1.0±0.5¶	0.8±0.4
BE, mmol/L				
NC	2.3±3.0	−23.4±1.2	−5.4±3.2	−4.9±3.5
O$_2$_CPR	2.7±2.7	−21.1±2.0*	−5.3±1.7	−4.3±1.8
O$_2$_CPR+postresuscitation	2.3±2.6	−20.9±2.1[†]	−3.1±2.2*	−3.1±1.6
O$_2$_postresuscitation	2.0±3.1	−23.1±2.1[‡,¶,*]	−3.1±2.4*	−3.3±1.6
TCO$_2$, mmol/L				
NC	27.3±3.5	8.6±1.8	19.4±2.6	20.8±4.0
O$_2$_CPR	28.3±2.8	11.9±3.1[†]	20.4±2.2	21.2±1.6
O$_2$_CPR+postresuscitation	28.0±3.4	12.0±2.6[†]	23.2±2.5[†,‡,*]	21.8±2.1
O$_2$_postresuscitation	28.3±2.7	8.0±2.0[§,¶]	22.8±1.4[†,§]	22.6±1.9

Data are shown as mean±SD. n=16 at each time point except for n=15 at PR180 and n=13 at PR360 in the NC group. PR5, PR180 and PR360: 5, 180 and 360 min postresuscitation; BE indicates base excess; CPR, cardiopulmonary resuscitation; NC, normoxic control; O$_2$_CPR, ventilated with 100% O$_2$ during CPR; O$_2$_CPR+postresuscitation, ventilated with 100% O$_2$ during CPR and the first 3 hours of postresuscitation; O$_2$_postresuscitation, ventilated with 100% O$_2$ during the first 3 hours of postresuscitation; and TCO$_2$, total carbon dioxide capacity.

*$P<0.05$ compared with NC.
†$P<0.01$ compared with NC.
‡$P<0.05$ compared with O$_2$_CPR.
§$P<0.01$ compared with O$_2$_CPR.
‖$P<0.05$ compared with O$_2$_CPR+postresuscitation.
¶$P<0.01$ compared with O$_2$_CPR+postresuscitation.

rarely observed in the sham group, but were frequently detected in the NC group. Fewer apoptotic neurons were observed in the CA1 region of the 3 hyperoxic ventilation groups compared with the NC group.

Representative photomicrographs of the immunohistochemical staining of DAPI/FJB in the CA1 region of the hippocampus from different animals in each group are shown in Figure 6. No FJB-positive neurons were noted in the sham group, whereas the NC group exhibited conspicuous degenerating neurons. Compared with the NC group, the proportion of the

FJB-positive area, the number of FJB-positive neurons, and the ratio of FJB-positive neurons were significantly reduced in the O$_2$_CPR+postresuscitation group (Figure 7).

Postresuscitation Oxidative Stress

SOD, MDA, and 8-OHDG level measurements of the hippocampus and cortex are shown in Figure 8. Compared with the sham-operated group, the hippocampus and cortex SOD levels were dramatically decreased, whereas the MDA and 8-OHDG levels were significantly increased

Table 3. Neurologic Deficit Score at 24, 48, 72, and 96 Hours After Resuscitation

Group	NC	O$_2$_CPR	O$_2$_CPR+Postresuscitation	O$_2$_Postresuscitation
24 h	435 (281–500)	320 (240–355)	252 (178–298)*	267 (233–312)
48 h	500 (213–500)	190 (175–285)	167 (137–210)*	210 (160–370)
72 h	500 (173–500)	140 (110–300)	123 (102–175)*	150 (110–327)
96 h	500 (136–500)	120 (95–235)	110 (75–151)*	132 (68–425)

Data are shown as median (IQR). n=16/group. NC indicates normoxic control; CPR, cardiopulmonary resuscitation; O$_2$_CPR, ventilated with 100% O$_2$ during CPR; O$_2$_CPR+postresuscitation, ventilated with 100% O$_2$ during CPR and the first 3 hours of postresuscitation; and O$_2$_postresuscitation, ventilated with 100% O$_2$ during the first 3 hours of postresuscitation.

*$P<0.05$ compared with NC.

in all of the experimental groups with the exception of hippocampus MDA, cortex MDA, and 8-OHDG levels in the O$_2$_CPR+postresuscitation group.

Compared with the NC group, hippocampus SOD levels in the O$_2$_CPR+postresuscitation group and cortex SOD levels in the 3 hyperoxygenation
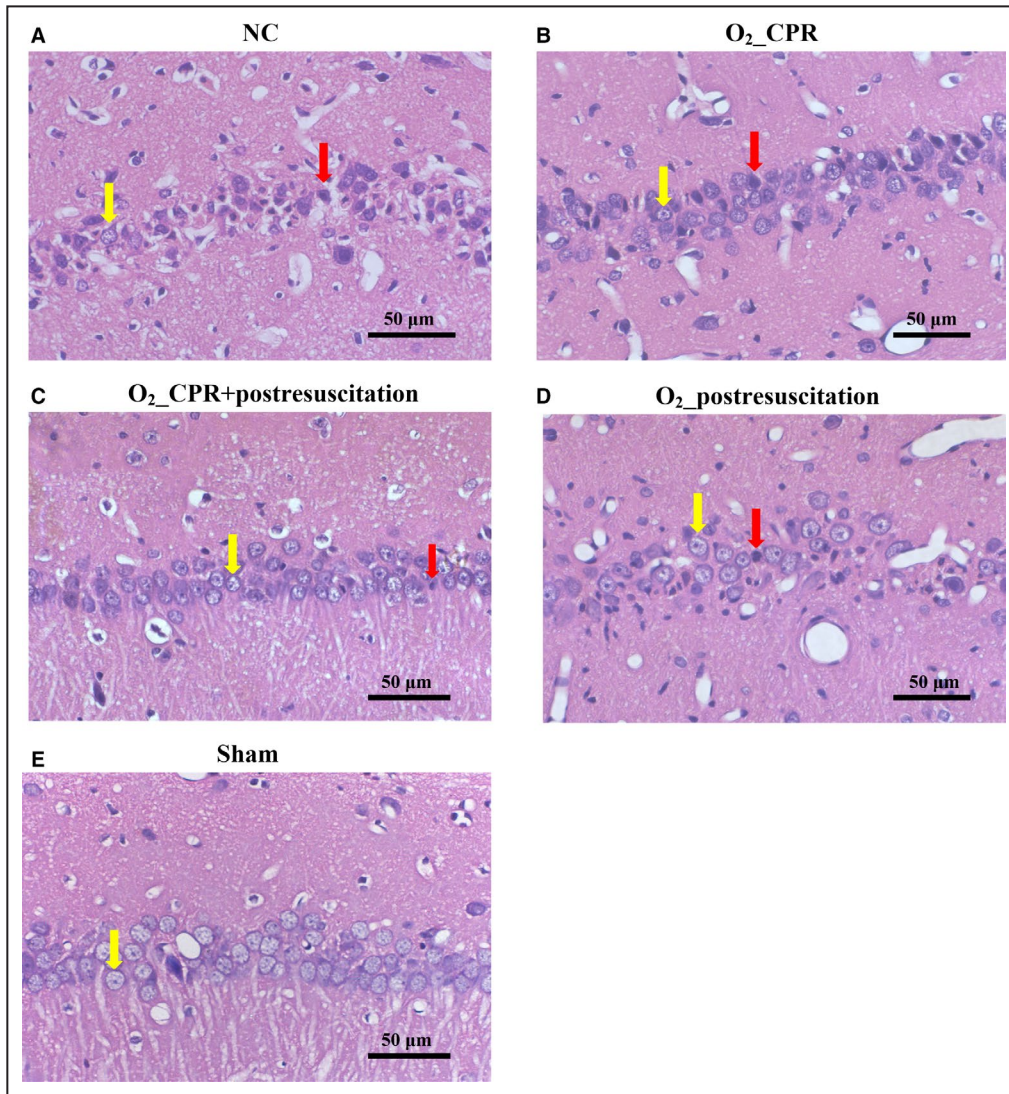

Figure 4. **Hematoxylin and eosin staining of neurons in the CA1 hippocampus region (n=6/group).**
Representative micrographs of the hematoxylin and eosin hippocampus CA1 at 96 hours after resuscitation in experimental and sham-operated animals (Sham) (**A** through **E**) (3 quantified fields/animal). Yellow arrows indicate viable neurons, and red arrows indicate abnormal neurons. CPR indicates cardiopulmonary resuscitation; NC, normoxic control; O$_2$_CPR, ventilated with 100% O$_2$ during CPR; O$_2$_CPR+postresuscitation, ventilated with 100% O$_2$ during CPR and the first 3 hours of postresuscitation; and O$_2$_postresuscitation, ventilated with 100% O$_2$ during the first 3 hours of postresuscitation.
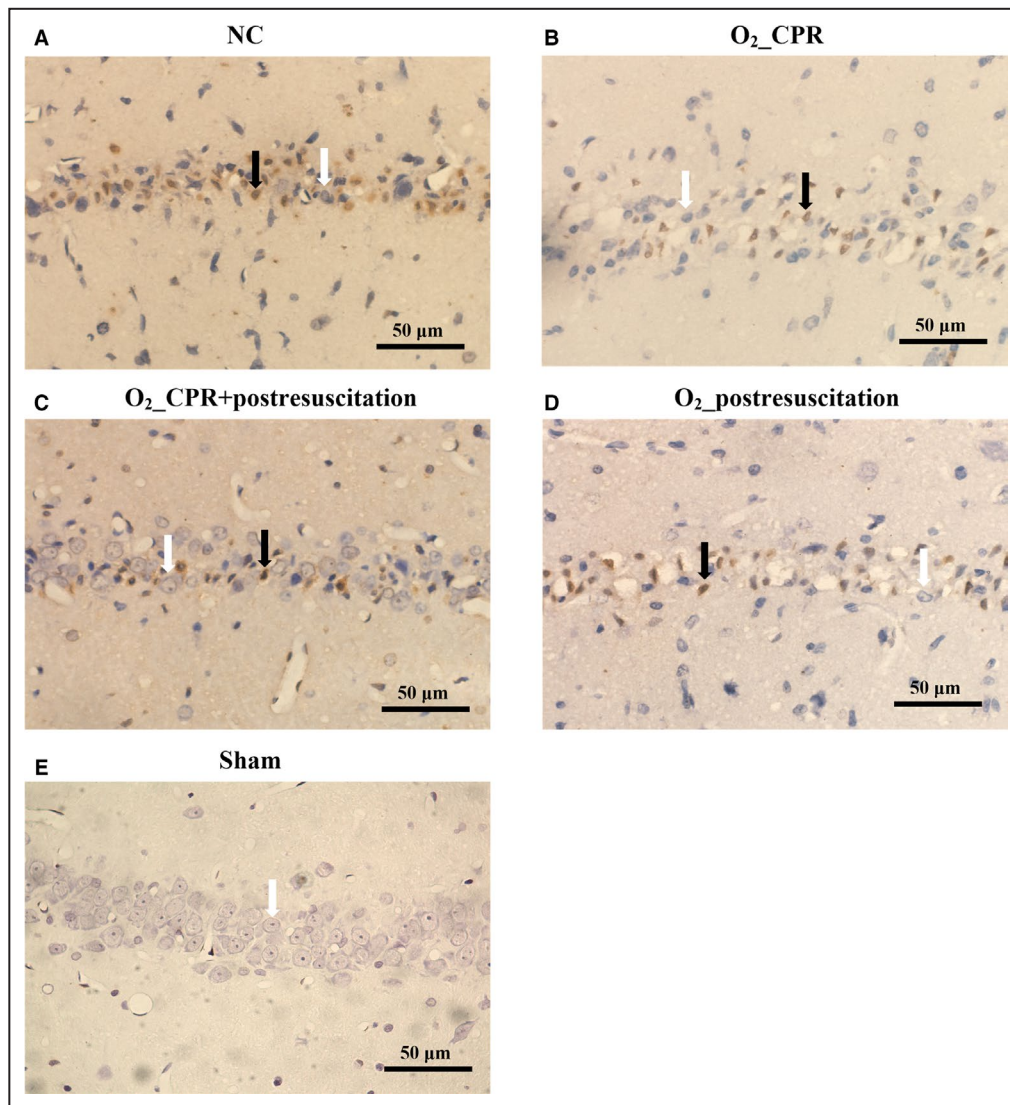
Figure 5. TUNEL (terminal deoxynucleotidal transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling) staining of neurons in the CA1 hippocampus region (n=6/group).
Apoptosis expression detected using the TUNEL method in the hippocampus CA1 region at 96 hours after resuscitation in experimental and sham-operated animals (Sham) (**A** through **E**) (3 quantified fields/animal). White arrows indicate TUNEL-negative neurons, and black arrows indicate TUNEL-positive neurons. NC indicates normoxic control; O_2_CPR, ventilated with 100% O_2 during CPR; O_2_CPR+postresuscitation, ventilated with 100% O_2 during CPR and the first 3 hours of postresuscitation; and O_2_postresuscitation, ventilated with 100% O_2 during the first 3 hours of postresuscitation.

groups were significantly increased. MDA contents in the hippocampus and cortex were significantly reduced in the O_2_CPR+postresuscitation group, and hippocampus 8-OHDG levels in the 3 hyperoxygenation groups and cortex 8-OHDG levels in the O_2_CPR+postresuscitation group were markedly reduced. In addition, cortex MDA and 8-OHDG levels were dramatically reduced in the O_2_CPR+ postresuscitation group compared with the O_2_ postresuscitation group.

DISCUSSION

The present study investigated the effect of the different timings of hyperoxygenation on neurological outcomes and oxidative stress in a rat model of CA/CPR treated with TTM. Our results confirmed that a transient period of global ischemia and reperfusion results in oxidative damage in brain tissue. Hyperoxygenation with TTM not only improved myocardial function but also attenuated cerebral injuries. High-concentration
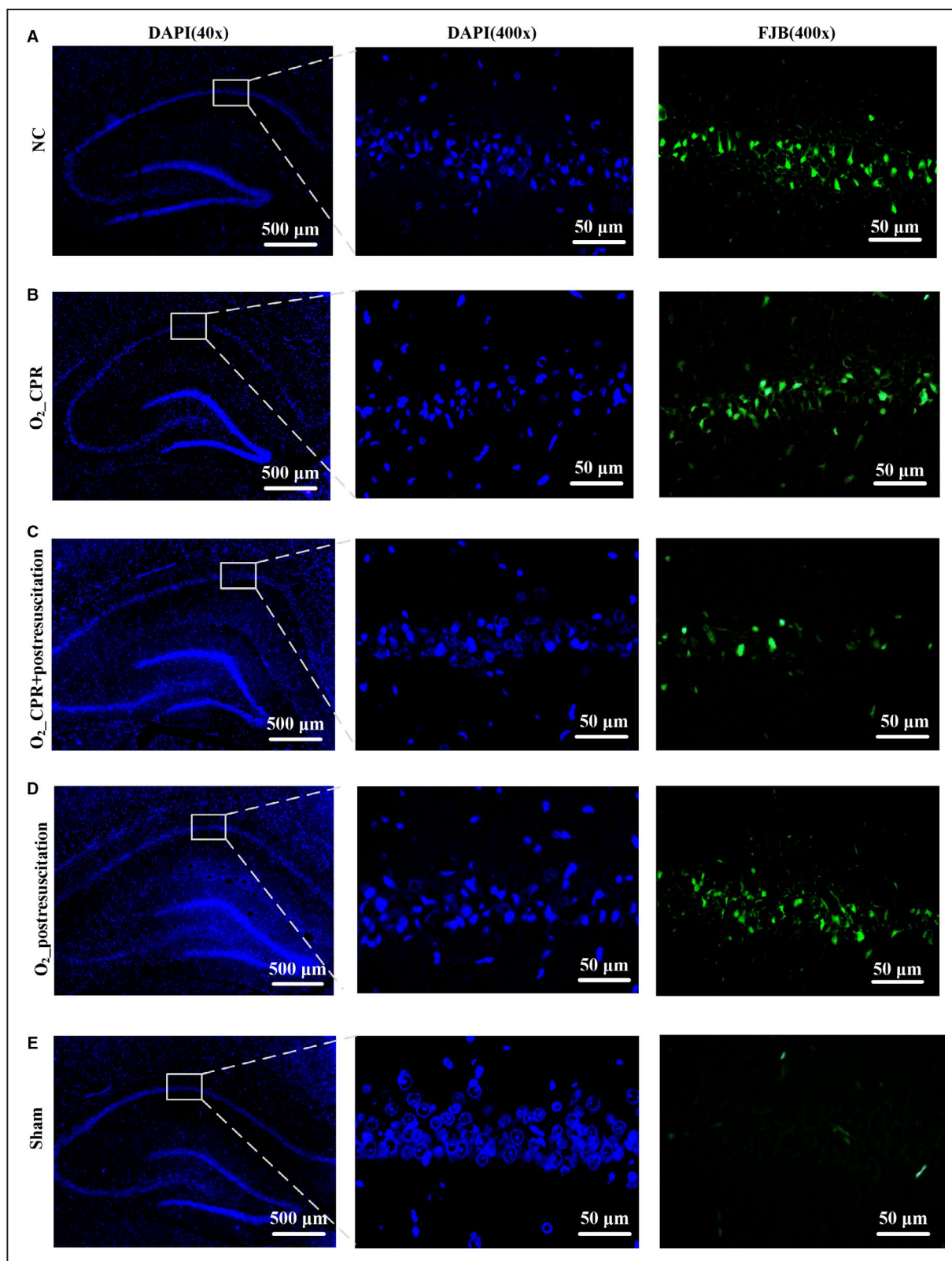
Figure 6. **The DAPI/FJB staining of neurons in the CA1 hippocampus region (n=6/group).**
Representative micrographs of the DAPI/FJB staining of the CA1 hippocampus region at 96 hours after resuscitation in experimental and sham-operated animals (Sham) (**A** through **E**) (3 quantified fields/animal). CPR indicates cardiopulmonary resuscitation; DAPI, 4′,6-diamidino-2-phenylindole; FJB, fluoro-jade B; NC, normoxic control; O_2_CPR, ventilated with 100% O_2 during CPR; O_2_CPR+postresuscitation, ventilated with 100% O_2 during CPR and the first 3 hours of postresuscitation; and O_2_postresuscitation, ventilated with 100% O_2 during the first 3 hours of postresuscitation.
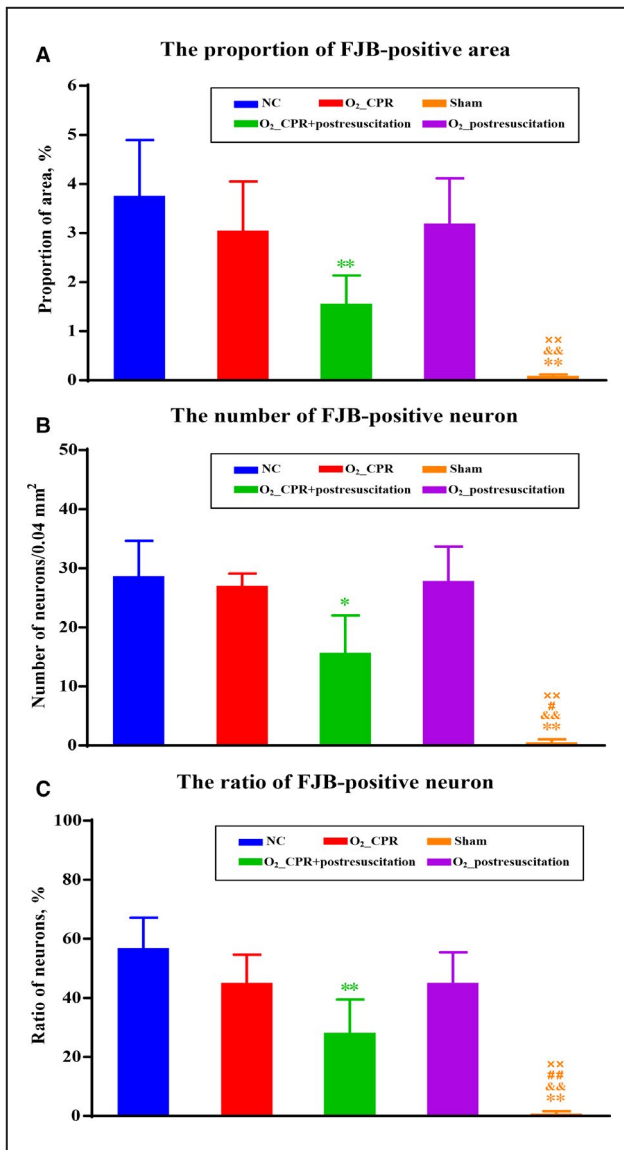
Figure 7. Semiquantitative analysis of FJB-positive neurons in the CA1 hippocampus region (n=6/group).
The proportion of the FJB-positive area (**A**), the number of FJB-positive neurons (**B**), and the ratio of FJB-positive neurons (**C**) were measured. CPR indicates cardiopulmonary resuscitation; FJB, fluoro-jade B; NC, normoxic control; $O_2\_$CPR, ventilated with 100% O_2 during CPR; $O_2\_$CPR+postresuscitation, ventilated with 100% O_2 during CPR and the first 3 hours of postresuscitation; and $O_2\_$postresuscitation, ventilated with 100% O_2 during the first 3 hours of postresuscitation. *$P<0.05$ compared with NC; **$P<0.01$ compared with NC; &&$P<0.01$ compared with $O_2\_$CPR; #$P<0.05$ compared with $O_2\_$CPR+postresuscitation; ##$P<0.01$ compared with $O_2\_$CPR+postresuscitation; ××$P<0.01$ compared with $O_2\_$postresuscitation.

oxygen ventilation was administered during CPR and postresuscitation hypothermia demonstrated significantly improved neurological recovery.

Ischemia/hypoxia and reperfusion/reoxygenation stimulates the increase in ROS, which may disturb the balance between oxidant and antioxidant mechanisms and play a central role in initiating and enhancing post-CA damage.[27] Hyperoxygenation may reduce hypoxia-related damage by improving tissue oxygen delivery, but may simultaneously increase the risk of an excessive production of ROS.[28] Ventilation with lower concentrations of oxygen during CPR is a potential strategy to ameliorate the oxidative stress associated with reperfusion. However, animal studies demonstrated that both hyperoxic ventilation ($FiO_2>0.5$) and normoxic ventilation ($FiO_2=0.21$) resulted in significantly improved ROSC rates compared with hypoxic ventilation ($FiO_2<0.21$), whereas no statistically discernible differences in resuscitation outcome and markers of oxidant injury were noted between hyperoxic and normoxic ventilations.[4,29–32] Consistent with previous studies, our results indicated that hyperoxygenation coupled with CPR did not compromise ROSC. After ROSC is achieved, persistent inadequate tissue oxygen delivery will cause ongoing tissue ischemia and prolong ischemic/reperfusion injury. As an important component of postresuscitation care, the optimal oxygenation management remains undetermined. On one hand, hyperoxygenation results in increased MAP, reduced metabolic acidosis, and improved myocardial and neurological recovery.[31,33,34] On the other hand, hyperoxygenation was also shown to exacerbate postischemic organ dysfunction as evidenced by increased oxidative stress, worsened neurological outcome, and decreased survival and degree of necrotic neurons.[7,8,35,36] Unfortunately, Angelos et al[37] reported that hypoxic reperfusion can also increase oxygen radical generation after resuscitation. Therefore, providing adequate oxygen delivery but limiting the oxidative stress represents a strategy to achieve a better recovery and to improve the long-term outcome.

TTM, or mild hypothermia, is one of the most persuasive interventions that has proven benefits for both the heart and the brain when commenced early after ROSC.[15] Characterized by transient and reversible global myocardial dysfunction, myocardial stunning is a component of post-CA syndrome and has recently been recognized as a leading cause for early death after successful resuscitation.[38] In the present study, myocardial stunning, cardiac injury, and oxidative stress were significantly ameliorated when hyperoxygenation was administered during TTM. The combined effects of hyperoxic ventilation and TTM could play an important role in the protection of myocardial function. Piao et al[39] showed that myocardial stunning was associated with increased mitochondrial ROS generation, whereas suppression of ROS production with specific inhibitors attenuated myocardial stunning and improved outcomes in mice. Zhao et al[31] and Hackenhaar et al[40] demonstrated that
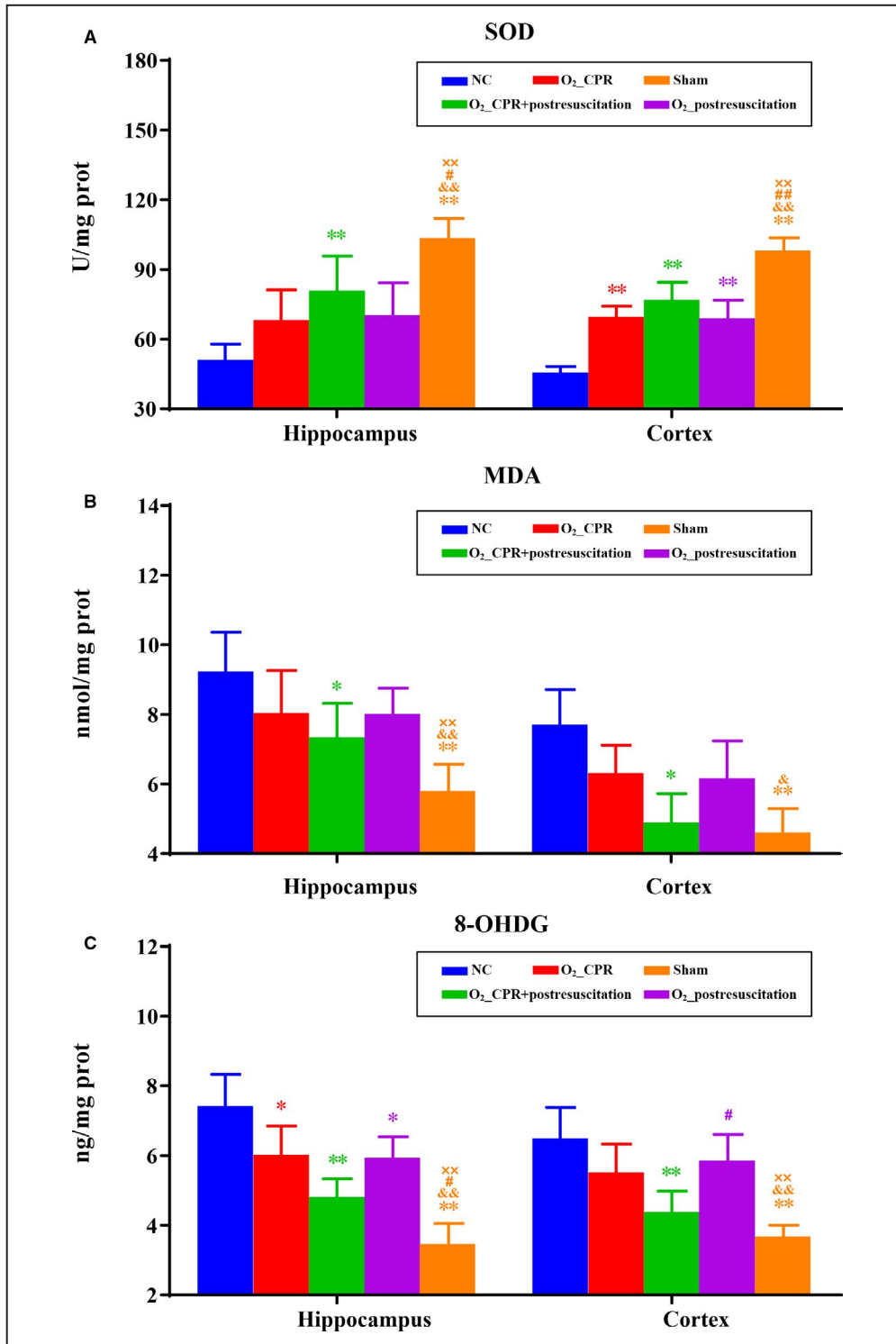
Figure 8. Oxidative stress marker levels in the hippocampus and cortex (n=6/group).
A, SOD, (**B**) MDA, and (**C**) 8-OHDG contents in the hippocampus and cortex were measured 6 hours after resuscitation in experimental and sham-operated animals (Sham). CPR indicates cardiopulmonary resuscitation; 8-OHDG, 8-hydroxy-2-deoxy guanosine; MDA, malondialdehyde; NC, normoxic control; O_2_CPR, ventilated with 100% O_2 during CPR; O_2_CPR+postresuscitation, ventilated with 100% O_2 during CPR and the first 3 hours of postresuscitation; O_2_postresuscitation, ventilated with 100% O_2 during the first 3 hours of postresuscitation; and SOD, superoxide dismutase. *$P<0.05$ compared with NC; **$P<0.01$ compared with NC; &$P<0.05$ compared with O_2_CPR; &&$P<0.01$ compared with O_2_CPR; #$P<0.05$ compared with O_2_CPR+postresuscitation; ##$P<0.01$ compared with O_2_CPR+postresuscitation; ××$P<0.01$ compared with O_2_postresuscitation.

high-concentration oxygen ventilation and mild hypothermia contributed to reduced oxidative damage, respectively. Therefore, mild hypothermia, which exerts protective effects after ROSC by decreasing oxidative stress products, restored adequate systemic hemodynamics by neutralizing the excessive ROS produced when hyperoxic reperfusion is applied.[41]

Brain injury is another component of post-CA syndrome and a common cause of morbidity and mortality.[42] The mismatch between cerebral blood flow and oxygen requirements of the ischemic tissue promotes excessive ROS formation and results in neuronal necrosis and apoptosis. The brain is particularly sensitive to oxidative stress given its high oxygen consumption, high content of intracellular-free iron, and low levels of enzymatic antioxidants. Mendoza-Paredes et al[34] and Solås et al[43] reported that the cerebrum was better protected when reoxygenation with 100% oxygen in piglets. Inconsistent with Mendoza-Paredes and Solås, Faa et al[44] reported a higher percentage of apoptotic neurons in piglets reoxygenated with 100% O_2. Tataranno et al[36] reported that resuscitating preterm infants with 100% oxygen was associated with higher oxidative stress compared with room air. The discrepancy in the effects of hyperoxygenation suggested that oxygen alone was not sufficient to prevent all of the deleterious consequences of brain ischemia. In the current study, we demonstrated that hyperoxygenation coupled with TTM greatly facilitated the neurological outcome as indicated by the improved electroencephalogram activity, attenuated cerebral injury, suppressed ROS production, and decreased neuronal degeneration ratio. In addition to its effect on ROS suppression, the neuroprotective actions of mild hypothermia are also related to pathways involving cerebral hemodynamics, energetics and metabolism, and apoptosis.[41] The improved hemodynamic stability and myocardial function together with improved cerebral blood flow and attenuated brain injury resulted in better neurological outcomes when hyperoxygenation is administered during TTM.[45–47]

This is the first study to investigate the timing effects of hyperoxic ventilation in association with TTM in a CA/CPR model. We found that hyperoxygenation with CPR and TTM greatly improve outcomes by decreasing ROS production, facilitating cardiac function, and promoting brain recovery. Given the limited clinical data on the effect of different timing of hyperoxic ventilation in patients resuscitated from CA, we designed 4 experimental groups to mimic patient resuscitation in different scenarios: victims resuscitated with/without 100% O_2 supply and treated with/without 100% O_2 after resuscitation by bystanders, paramedics, or physicians. Our results suggest that whether started at CPR or started at postresuscitation, hyperoxic ventilation should be coupled with TTM.

Some limitations have to be taken into account in our study. First, the animal models did not imitate the clinical scenario of CA completely given that the study was performed in healthy animals with no previous myocardial damage and the mechanism of CA was only electrical. Second, this study did not compare the effects of different oxygen concentrations other than 100% O_2 on oxidative stress and neurological outcomes, and the optimal oxygen concentration administration for patients resuscitated from CA remains undetermined. Third, the animals in our study were treated with the same selected target temperature and duration for therapeutic hypothermia, the potential interaction mechanism between hyperoxic ventilation and TTM merits further investigation. Fourth, activation of astrocytes and microglia in the hippocampus and cerebral cortex is also a marker of brain ischemia and reperfusion injury, but these markers were not evaluated in our experiments.

CONCLUSIONS

In this CA/CPR rat model, hyperoxygenation coupled with TTM attenuates ischemia/reperfusion-induced injuries and improves survival rate. The beneficial effects of high-concentration oxygen ventilation are timing and duration dependent. Hyperoxygenation commenced with CPR and administered during hypothermia after resuscitation improves outcomes.

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