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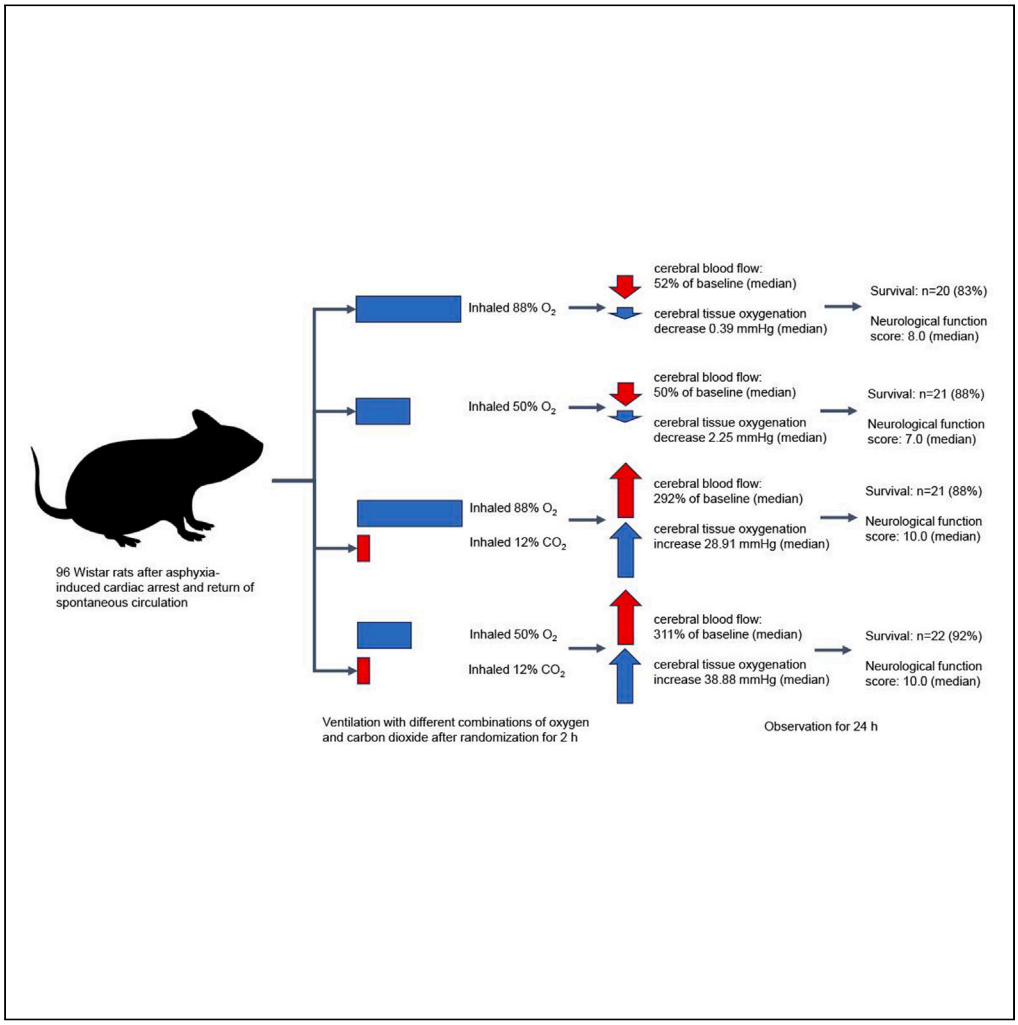

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shinghwaliu@ntu.edu.tw (S.-H.L.)
wjchen1955@ntu.edu.tw (W.-J.C.)

Highlights

Post-arrest cerebral blood flow and tissue oxygenation are mainly regulated by CO_2

Reducing inhaled O_2 could not effectively mitigate post-arrest cerebral injuries

Inhaled CO_2 reduced hyperoxic injuries, facilitating neurological recovery

Inhalation of 50% O_2 and 12% CO_2 was most neuroprotective

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Optimal inhaled oxygen and carbon dioxide concentrations for post-cardiac arrest cerebral reoxygenation and neurological recovery

Chih-Hung Wang,[1,2] Wei-Tien Chang,[1,2] Chien-Hua Huang,[1,2] Min-Shan Tsai,[1,2] Chan-Chi Wang,[1,2] Shing-Hwa Liu,[3,4,5,7,*] and Wen-Jone Chen[1,2,6,7,8,*]

SUMMARY

Prolonged cerebral hypoperfusion after the return of spontaneous circulation (ROSC) from cardiac arrest (CA) may lead to poor neurological recovery. In a 7-min asphyxia-induced CA rat model, four combinations of inhaled oxygen (iO_2) and carbon dioxide (iCO_2) were administered for 150 min post-ROSC and compared in a randomized animal trial. At the end of administration, the partial pressure of brain tissue oxygenation ($PbtO_2$) monitored in the hippocampal CA1 region returned to the baseline for the 88% iO_2 [$\Delta PbtO_2$, median: −0.39 (interquartile range: 5.6) mmHg] and 50% iO_2 [$\Delta pbtO_2$, −2.25 (10.9) mmHg] groups; in contrast, $PbtO_2$ increased substantially in the 88% iO_2+12% iCO_2 [$\Delta pbtO_2$, 35.05 (16.0) mmHg] and 50% iO_2+12% iCO_2 [$\Delta pbtO_2$, 42.03 (31.7) mmHg] groups. Pairwise comparisons (*post hoc* Dunn's test) indicated the significant role of 12% iCO_2 in augmenting $PbtO_2$ during the intervention and improving neurological recovery at 24 h post-ROSC. Facilitating brain reoxygenation may improve post-CA neurological outcomes.

INTRODUCTION

Annually, out-of-hospital cardiac arrest (CA) occurs in approximately 44 individuals per 100,000 people worldwide.[1] Only 8% of patients subsequently recover their neurological function.[2] Brain injury was reported to account for 65% of deaths during post-CA intensive care.[3] Post-CA brain injury[4,5] is induced by a primary hypoxia-ischemia insult during CA and cardiopulmonary resuscitation (CPR), which is followed by secondary injuries after the return of spontaneous circulation (ROSC) caused by protracted cerebral hypoperfusion.[6–8]

The goal in treating CA is to restore cerebral oxygen delivery and tissue oxygenation as the brain is a highly energy-consuming organ[9] susceptible to hypoxic injuries.[10] Cerebral oxygen delivery is primarily determined by cerebral blood flow (CBF) and blood oxygen content. Several pathophysiologic factors[4,5] have been identified which may influence CBF after ROSC, including microcirculatory perturbation,[11,12] cerebral edema,[13] blood pressure,[8] and partial pressures of carbon dioxide ($PaCO_2$).[14] On the other hand, the oxygen content is determined by the amount of oxygen transported in circulation, including oxygen bound to hemoglobin and oxygen dissolved in the plasma, mainly controlled by partial pressures of arterial oxygen (PaO_2).[15] Several methods have been tested to recover CBF, including thrombolytics to improve cerebral microcirculation,[16] hypertonic saline to resolve cerebral edema,[13] and vasopressors to elevate blood pressure.[8] The success of these previous studies[8,13,16] suggested that recovering CBF and cerebral reoxygenation may help facilitate post-CA neurological recovery.

Among the pathophysiologic factors influencing cerebral oxygen delivery,[4,5] PaO_2 and $PaCO_2$ were recognized by the resuscitation guidelines from American Heart Association[17] and European Resuscitation Council[18] as the critical respiratory parameters which should be immediately managed after patients achieved ROSC. However, the optimal ranges of PaO_2 and $PaCO_2$ to recover cerebral oxygenation remain unknown.[17,18] Increasing concentration of oxygen inhalation to enhance the arterial oxygen content has been posited as a vital method to improve post-CA cerebral oxygen delivery.[5] Nonetheless, supranormal PaO_2 has been noted to decrease CBF,[19] leading to adverse effects.[20] On the other hand, cerebrovascular resistance has been noted to increase for at least 24 h in comatose survivors of CA.[21] $PaCO_2$ could modulate cerebrovascular resistance and CBF through its impact on vascular smooth muscle.[22] Notably, after hypoxia-ischemia brain injury, the reactivity of the vasculature to $PaCO_2$ remains preserved,[23] suggesting the possibility of carbon dioxide as a critical factor in determining cerebral oxygen delivery after ROSC.

[1]Department of Emergency Medicine, National Taiwan University Hospital, Taipei, Taiwan
[2]Department of Emergency Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan
[3]Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan
[4]Department of Medical Research, China Medical University Hospital, China Medical University, Taichung, Taiwan
[5]Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan
[6]Department of Internal Medicine, Min-Sheng General Hospital, Taoyuan, Taiwan
[7]These authors contributed equally
[8]Lead contact
*Correspondence: shinghwaliu@ntu.edu.tw (S.-H.L.), wjchen1955@ntu.edu.tw (W.-J.C.)
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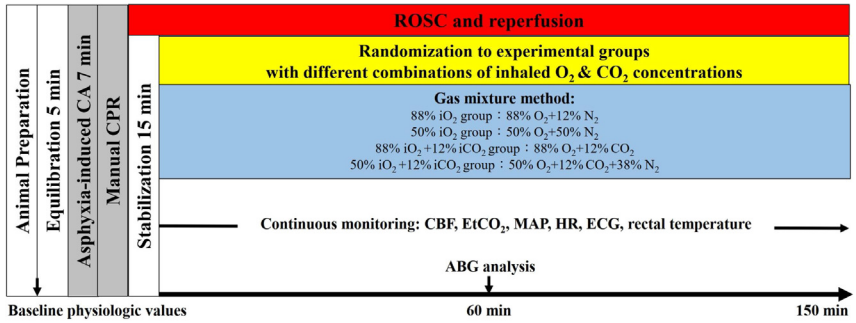

Figure 1. Study procedures and measurements at baseline, asphyxia, cardiac arrest, cardiopulmonary resuscitation, and reperfusion

ABG, arterial blood gas; CA, cardiac arrest; CBF, cerebral blood flow; CPR, cardiopulmonary resuscitation; ECG, electrocardiogram; $EtCO_2$, partial pressure of end-tidal carbon dioxide; HR, heart rate; MAP, mean arterial pressure; ROSC, return of spontaneous circulation.

In this study, we hypothesized that improving brain tissue reoxygenation may facilitate post-CA neurological recovery in an asphyxia-induced CA rat model. We assumed that adjusting the concentrations of inhaled oxygen (iO_2) and carbon dioxide (iCO_2) may augment cerebral reoxygenation after ROSC because of the effects of PaO_2 and $PaCO_2$ on CBF and oxygen content. We aimed to compare the effects of different combinations of iO_2 and iCO_2 concentrations on CBF, brain tissue oxygenation, and neurological outcomes to identify the optimal use of iO_2 and iCO_2 in post-ROSC care.

RESULTS

Baseline characteristics and resuscitation variables indicate successful randomization

Following the experimental protocol (Figure 1), 140 rats were used for the experiments, 96 of which were randomized to the study groups (Figure S1). Seven animals died before intervention completion, possibly caused by post-CA myocardial dysfunction.[5,24] Table 1 shows the baseline characteristics and resuscitation variables among the groups. The CPR time is noted to be numerically shorter in the 50% iO_2+12% iCO_2 (median: 88 s, interquartile range [IQR]:76.3s) group than the 50% iO_2 (135s, IQR:162s) group. Nonetheless, the scatterplot (Figure S2) demonstrates no significant statistical differences between the 50% iO_2+12% iCO_2 and the 50% iO_2 groups.

Arterial blood gas (ABG) analysis observes the hyperoxia and hypercapnia caused by iO_2 and iCO_2

At 60 min post-ROSC, ABG analysis shows that PaO_2 was above and below 300 mmHg in the groups administered 88% iO_2 and 50% iO_2, respectively (Table 1; Figure S3). For groups receiving 12% iCO_2, the median $PaCO_2$ was approximately 146 mmHg. Interestingly, although not receiving 12% iCO_2, the 88% iO_2 and 50% iO_2 groups demonstrate that the median $PaCO_2$ levels are 65.4 and 58.1 mmHg, respectively.

12% iCO_2 increases CBF and $PbtO_2$ regardless of the iO_2 concentrations

As shown in Figure 2, and Table 1, in the 88% iO_2 and 50% iO_2 groups, after the initial hyperemic state, CBF decreases to 52% (IQR: 13%) and 50% (IQR: 16%) of baseline at 150 min post-ROSC, respectively. In contrast, in the 88% iO_2+12% iCO_2 and 50% iO_2+12% iCO_2 groups, CBF at 150 min post-ROSC are 292% (IQR: 43%) and 311% (IQR: 102%) of baseline, respectively. Along with CBF, partial pressure of brain tissue oxygenation ($PbtO_2$) increases and decreases in groups with and without 12% iCO_2 administration, respectively. At 150 min post-ROSC, the $\Delta PbtO_2$, defined as $PbtO_2$ at a particular time point minus the baseline, suggests that $PbtO_2$ almost returns to the baseline value for 88% iO_2 [−0.39 (5.6) mmHg] and 50% iO_2 [−2.25 (10.9) mmHg] groups; in contrast, the $\Delta PbtO_2$ data suggests that $PbtO_2$ increases substantially from the baseline in the 88% iO_2+12% iCO_2 [35.05 (16.0) mmHg] and 50% iO_2+12% iCO_2 [42.03 (31.7) mmHg] groups. Pairwise comparisons indicate that the between-group differences in CBF, $PbtO_2$, and $\Delta PbtO_2$ are mainly caused by 12% iCO_2. For end-tidal carbon dioxide ($EtCO_2$), Figure S4 suggests that the ventilation status or the $PaCO_2$ level may become stable after 30 min post-ROSC. In contrast, after 30 min post-ROSC, the CBF and $PbtO_2$ continue to increase in the groups receiving 12% iCO_2 (Figure 2). As for hemodynamics, Figure S4 reveals that different combinations of iO_2 and iCO_2 did not influence blood pressure. Also, Figure S4 demonstrates no significant differences in body temperature, which was controlled closely around the target temperature.

12% iCO_2 improves neurological recovery without influencing survival

Figure 3 and Table 1 demonstrate no differences in survival among the groups. For rats surviving 24 h, neurological function score (NFS) is significantly higher in the groups administered 12% iCO_2 than in the groups without it. Figure 4 and Table 1 reveal that, compared with the groups without 12% iCO_2 administration, the groups receiving 12% iCO_2 have lower neuron-specific enolase (NSE) and malondialdehyde (MDA) concentrations, suggesting fewer neuronal and oxidative injuries in the latter groups. In contrast, there are no significant differences in cardiac troponin I, suggesting that 12% iCO_2 administration may not cause myocardial injuries.

Table 1. Baseline characteristics, peri-resuscitation variables, and outcomes of randomized animals

Variables	88% iO_2 (n = 24)	50% iO_2 (n = 24)	88% iO_2 + 12% iCO_2 (n = 24)	50% iO_2 + 12% iCO_2 (n = 24)	p value[a]
Baseline characteristics and resuscitation variables					
Body weight, (g)	465 (47.5)	475 (26.3)	465 (51.3)	478 (41.3)	0.76
Cardiac arrest time, (s)	198 (30.3)	201 (20.3)	209 (27.8)	203 (27.8)	0.43
CPR time, (s)	113 (72)	135 (162)	116 (90.3)	88 (76.3)	0.33
ABG analysis at 60 min post-ROSC					
pH	7.27 (0.04) (n = 20)	7.28 (0.07) (n = 24)	7.03 (0.07) (n = 22)	7.05 (0.05) (n = 23)	<0.001
$PaCO_2$, mmHg	65.4 (5.2) (n = 20)	58.1 (6.6) (n = 24)	146.9 (11.0) (n = 22)	146.3 (13.0) (n = 23)	<0.001
PaO_2, mmHg	356.8 (43.2) (n = 20)	174.4 (71.6) (n = 24)	430.2 (79.5) (n = 22)	209.3 (112.5) (n = 23)	<0.001
HCO_3^-, mEq/L	31.7 (1.6) (n = 20)	30.7 (5.5) (n = 24)	39.4 (3.0) (n = 22)	39.8 (7.8) (n = 23)	<0.001
Base excess, mEq/L	4.9 (1.3) (n = 20)	3.4 (4.5) (n = 24)	9.0 (3.9) (n = 22)	9.5 (6.2) (n = 23)	<0.001
Lactate, mg/dL	10.6 (4.9) (n = 20)	8.4 (8.7) (n = 24)	11.3 (3.4) (n = 22)	4.6 (5.3) (n = 23)	0.001
Physiological parameters at 150 min post-ROSC					
CBF level, % of baseline	0.52 (0.13) (n = 20)	0.50 (0.16) (n = 24)	2.92 (0.43) (n = 22)	3.11 (1.02) (n = 23)	<0.001
$PbtO_2$, mmHg	12.87 (15.0) (n = 20)	8.33 (11.0) (n = 24)	35.05 (16.0) (n = 22)	42.03 (31.7) (n = 23)	<0.001
$\Delta PbtO_2$, mmHg	−0.39 (5.6) (n = 20)	−2.25 (10.9) (n = 24)	28.91 (23.2) (n = 22)	38.88 (35.7) (n = 23)	<0.001
$EtCO_2$, mmHg	60.5 (4.0) (n = 20)	51.0 (5.0) (n = 24)	138.5 (5.0) (n = 22)	131 (4.0) (n = 23)	<0.001
MAP, mmHg	98.3 (25.0) (n = 20)	100.4 (25.8) (n = 24)	97.2 (25.5) (n = 22)	101.2 (24.4) (n = 23)	>0.99
Body temperature, °C	36.8 (0.1) (n = 20)	36.9 (0.2) (n = 24)	36.9 (0.2) (n = 22)	36.8 (0.3) (n = 23)	0.21
ELISA and TBARS at 24 h post-ROSC					
NSE level at 24 h, ng/ml	0.663 (0.336) (n = 20)	0.828 (0.453) (n = 21)	0.351 (0.119) (n = 21)	0.305 (0.103) (n = 22)	<0.001
S100β level, pg/ml	1.461 (0.284) (n = 20)	1.562 (0.297) (n = 21)	1.397 (0.123) (n = 21)	1.404 (0.082) (n = 22)	0.03
MDA level, μM	93.2 (50.0) (n = 20)	91.3 (19.3) (n = 21)	59.0 (59.8) (n = 21)	64.9 (48.3) (n = 22)	<0.001
Cardiac troponin I, ng/ml	72.1 (58.7) (n = 20)	90.5 (69.2) (n = 21)	42.6 (48.0) (n = 21)	39.7 (50.5) (n = 22)	0.002
TUNEL assay and FJC staining at 24 h post-ROSC					
Density of TUNEL-positive cells: CA1, count/mm^2	361.5 (179.2) (n = 6)	415.6 (142.8) (n = 6)	69.8 (15.6) (n = 6)	61.5 (24.5) (n = 6)	<0.001
Density of TUNEL-positive cells: CA3, count/mm^2	364.6 (79.7) (n = 6)	358.3 (108.9) (n = 6)	58.3 (21.9) (n = 6)	61.5 (29.2) (n = 6)	<0.001
Density of FJC-positive cells: CA1, count/mm^2	43.8 (16.2) (n = 6)	38.5 (6.8) (n = 6)	25.0 (7.3) (n = 6)	20.8 (10.4) (n = 6)	0.004
Density of FJC-positive cells: CA3, count/mm^2	29.2 (10.4) (n = 6)	34.4 (9.9) (n = 6)	25.0 (10.4) (n = 6)	18.8 (10.9) (n = 6)	0.05
Outcomes at 24 h post-ROSC					
Survival, n (%)	20 (83)	21 (88)	21 (88)	22 (92)	0.86[b]
Neurological function score	8.0 (1.0) (n = 20)	7.0 (1.0) (n = 21)	10.0 (1.0) (n = 21)	10.0 (0.0) (n = 22)	<0.001

Data are expressed as the median (interquartile range) or count (proportion). ABG, arterial blood gas; CBF, cerebral blood flow; CPR, cardiopulmonary resuscitation; $\Delta PbtO_2$, delta of partial pressure of brain tissue oxygenation; ELISA, enzyme-linked immunosorbent assay; $EtCO_2$, partial pressure of end-tidal carbon dioxide; FJC, Fluoro-Jade C; HCO_3^-, sodium bicarbonate; iCO_2, inhaled carbon dioxide; iO_2, inhaled oxygen MAP, mean arterial pressure; MDA, malondialdehyde; NSE, neuron-specific enolase; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; ROSC, return of spontaneous circulation; TBARS, thiobarbituric acid reactive substance; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

[a]The comparison was performed using Kruskal-Wallis test. The results of *post hoc* pairwise comparisons are annotated in corresponding figures.

[b]This comparison was performed using chi-squared test.

Figure 5 indicates lower densities of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells in the hippocampal CA1 and CA3 regions of the groups receiving 12% iCO_2, suggesting that apoptosis has been attenuated. In contrast, regarding neural degeneration, only the 50% iO_2+12% iCO_2 group exhibits a significantly lower density of Fluoro-Jade C (FJC)-positive cells than the 88% iO_2 and 50% iO_2 groups in the hippocampal CA1 region. No significant differences are observed in caspase-3 activation or PARP cleavage (Figure S5).

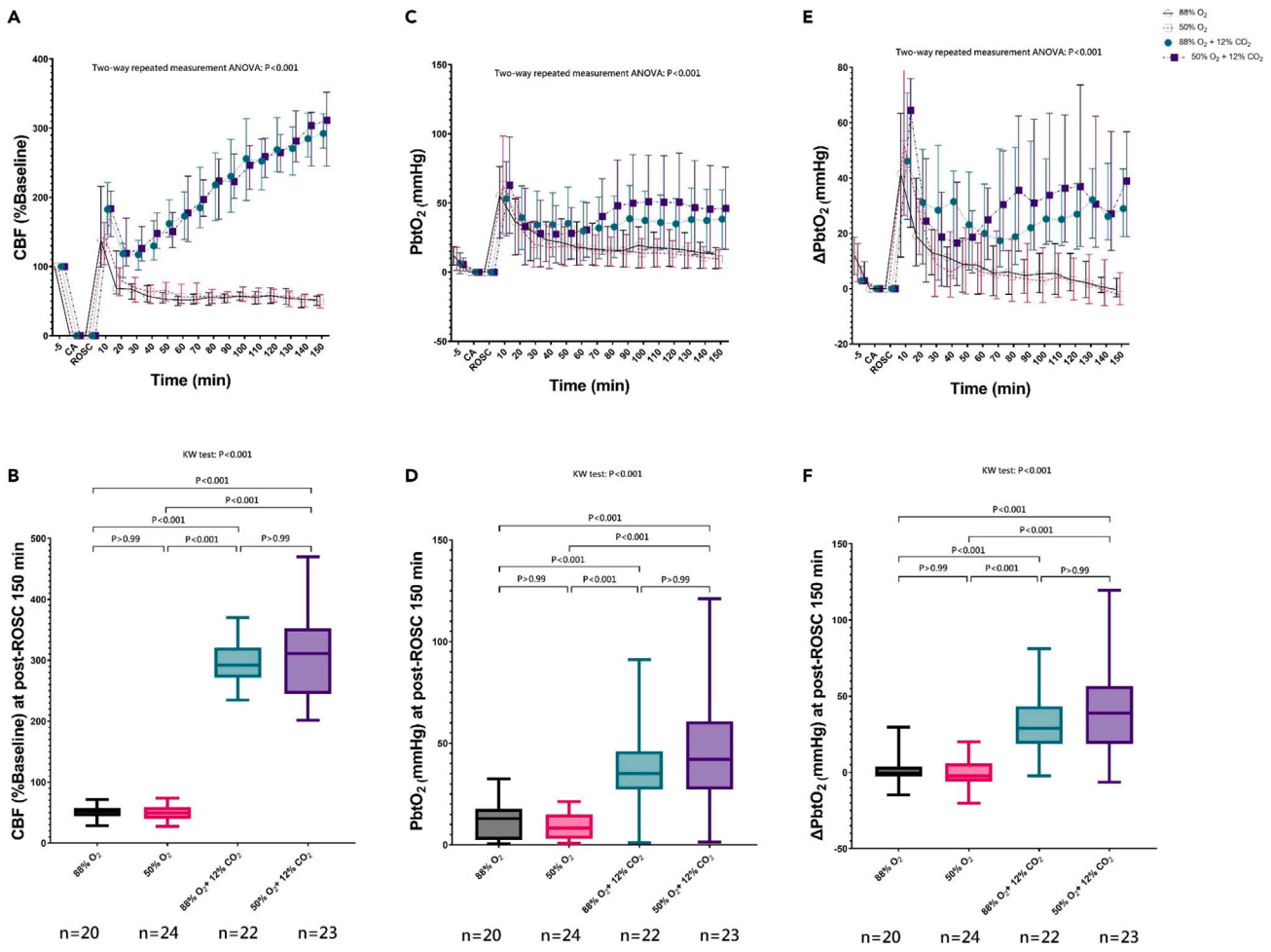

Figure 2. Brain oxygenation during invasive monitoring

(A), (C), and (E) Trends of CBF and $PbtO_2$ after administration of different combinations of iO_2 and iCO_2.

(B), (D), and (F) Levels of CBF and $PbtO_2$ at the end of the intervention, 150 min post-ROSC. Black: 50% iO_2 group; pink: 88% iO_2 group; green: 50% iO_2 + 12% iCO_2 group; purple: 88% iO_2 + 12% iCO_2 group. The administration of 12% iCO_2 increased CBF and $PbtO_2$. Data are presented as the median with an interquartile range or a boxplot. Pairwise comparisons of physiological parameters between experimental groups were performed by *post hoc* Dunn's test.

CA, cardiac arrest; CBF, cerebral blood flow; $\Delta PbtO_2$, delta of partial pressure of brain tissue oxygenation; iCO_2, inhaled carbon dioxide; iO_2, inhaled oxygen; KW: Kruskal-Wallis; ROSC, return of spontaneous circulation.

Sensitivity analysis suggests 50% iO_2+12% iCO_2 might be the optimal combination

The sensitivity analysis reveals significant interactions between iO_2 and iCO_2 in NFS ($p < 0.001$) (Figure S6). *Post hoc* analysis suggests that the 50% iO_2+12% iCO_2 and 50% iO_2 groups demonstrate the highest and lowest NFS, respectively, among all combinations of iO_2 and iCO_2.

DISCUSSION

Main findings

First, post-CA CBF and $PbtO_2$ were mainly modulated by 12% iCO_2 rather than iO_2. Second, reducing the iO_2 concentration from 88 to 50% could not effectively mitigate post-ROSC cerebral injuries. In contrast, 12% iCO_2 reduced the severity of hyperoxic injuries and facilitated neurological recovery. Third, the improved neurological outcomes could be explained by fewer neuronal injuries, reduced oxidative injuries, attenuated apoptosis, and decreased neuronal degeneration associated with 12% iCO_2. Finally, there was a significant interaction between iO_2 and iCO_2, suggesting that 50% iO_2 and 12% iCO_2 might be the most neuroprotective combination among all groups.

Determining iO_2 and iCO_2 concentrations and intervention duration

PaO_2 >300 mmHg and PaO_2 between 101 and 299 mmHg were categorized as severe and moderate hyperoxia, respectively.[25] In this study, both groups administered 88% iO_2 achieved median PaO_2 above 300 mmHg (Table 1). PaO_2 >300 mmHg was reported to be

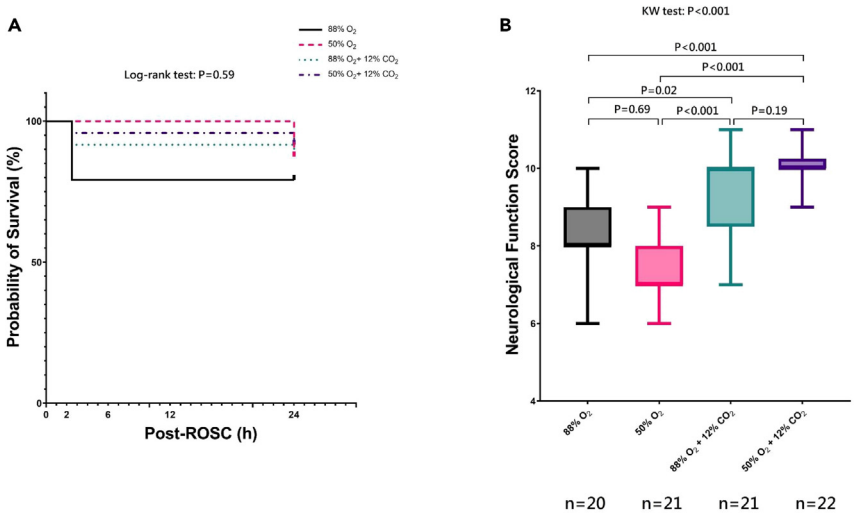

Figure 3. Survival curves and neurological outcomes at 24 h post-ROSC

(A) Kaplan-Meier survival curves.

(B) Neurological function scores at 24 h post-ROSC. Black: 50% iO_2 group; pink: 88% iO_2 group; green: 50% iO_2 + 12% iCO_2 group; purple: 88% iO_2 + 12% iCO_2 group. The neurological function score is presented as a boxplot. Pairwise comparisons between experimental groups were performed using *post hoc* Dunn's test. There were no significant differences in survival among experimental groups. The neurological function score was higher in groups with 12% iCO_2 than in groups without it. iCO_2, inhaled carbon dioxide; iO_2, inhaled oxygen; KW, Kruskal-Wallis; ROSC, return of spontaneous circulation.

associated with increased post-CA mortality.[26] In contrast, the median PaO_2 of groups receiving 50% iO_2 was 174 and 209 mmHg (Table 1). Conflicting findings about the association of PaO2 between 101 and 299 mmHg with poor post-CA outcomes have been reported.[27–31] A prospective observational study[32] indicated that the association of PaO_2 with poor neurological outcome began at ≥ 300 mmHg. Meanwhile, an animal study[33] demonstrated that 24 h survival was higher in a group receiving 50% iO_2 (survival: 5/8, 62.5%) than in those receiving 21% (survival: 1/7, 14.3%) or 100% iO_2 (survival: 4/8, 50%) in a ventricular fibrillation (VF)-induced CA rat model. Therefore, from clinical and experimental perspectives, it may be reasonable to hypothesize that, compared with 88% iO_2, 50% iO_2 would improve neurological outcomes.

As for the selection of iCO_2 concentration, no relevant clinical studies could be used for reference. In a prospective observational study, Kilgannon et al.[34] indicated that a mean $PaCO_2$ of 68 mmHg was associated with the highest chances of good neurological outcome. In the CCC trial, Eastwood et al.[35] compared $PaCO_2$ of 50–55 mmHg versus 35–45 mmHg in post-ROSC patients, revealing a lower NSE level in the former. Our previous dose-response study[14] tested and compared the effects of 4%, 8%, and 12% iCO_2 on CBF and neuroprotection. The results revealed significant linear trends between increasing iCO_2 concentrations and neuroprotective effects. Therefore, we selected 12% iCO_2 to maximize the neuroprotective effects, ensuring the hyperoxia injuries could be counteracted. Interestingly, the median $PaCO_2$ in 88% and 55% iO_2 groups were 65.4 and 58.1 mmHg, respectively, already achieving the $PaCO_2$ levels considered neuroprotective.[34,35] In a randomized clinical trial, Bernard et al.[36] demonstrated that the median $PaCO_2$ levels measured approximately 1 h after ROSC was 57 mmHg for patients resuscitated from out-of-hospital CA. The $PaCO_2$ levels noted in the Bernard et al.[36] trial were quite similar to the $PaCO_2$ levels measured at 60 min post-ROSC and $EtCO_2$ levels at 150 min post-ROSC in the 88% iO_2 and 50% iO_2 groups (Table 1). During the immediate post-ROSC period, cerebral injuries-associated depressed consciousness may cause hypoventilation and elevated $PaCO_2$.[37] Therefore, the $PaCO_2$ or $EtCO_2$ levels in the 88% iO_2 and 50% iO_2 groups may reflect the clinical scenarios. Furthermore, in our experiments, the pentobarbital administered during animal preparation may also induce respiratory depression and lead to elevated $PaCO_2$ and $EtCO_2$ levels. If lower iCO_2 concentrations had been used, the neuroprotective effects could have been insignificant because of the slight between-group differences in $PaCO_2$.

In an asphyxia-induced CA rat model, Drabek et al.[6] noted that after the initial hyperemia phase peaking at post-ROSC 7 min, a significant hypoperfusion phase ensued since post-ROSC 20 min till 60 min. Using a VF-induced CA rat model, Gong et al.[7] observed a sustained decrease of cerebral cortex microcirculation for 8 h following ROSC. For out-of-hospital CA, clinical trials[36,38] demonstrated that the interval between ROSC and ABG examination was approximately 90–150 min. After 90–150 min, clinicians may adjust iO_2 concentrations according to ABG results or other monitoring facilities without sticking to a predetermined inhalation concentration. Therefore, the selecting intervention duration to be 150 min may be reasonable.

Influence of different iO_2 or iCO_2 concentrations on brain reoxygenation

In the 88% iO_2 group (Figures 2A and 2B), after initial hyperemia, CBF levels continued to decrease, indicating the "no-reflow" phenomenon.[39,40] Breathing high-concentration O_2 could cause significant cerebral vasoconstriction.[41] Nonetheless, reducing the iO_2 concentration

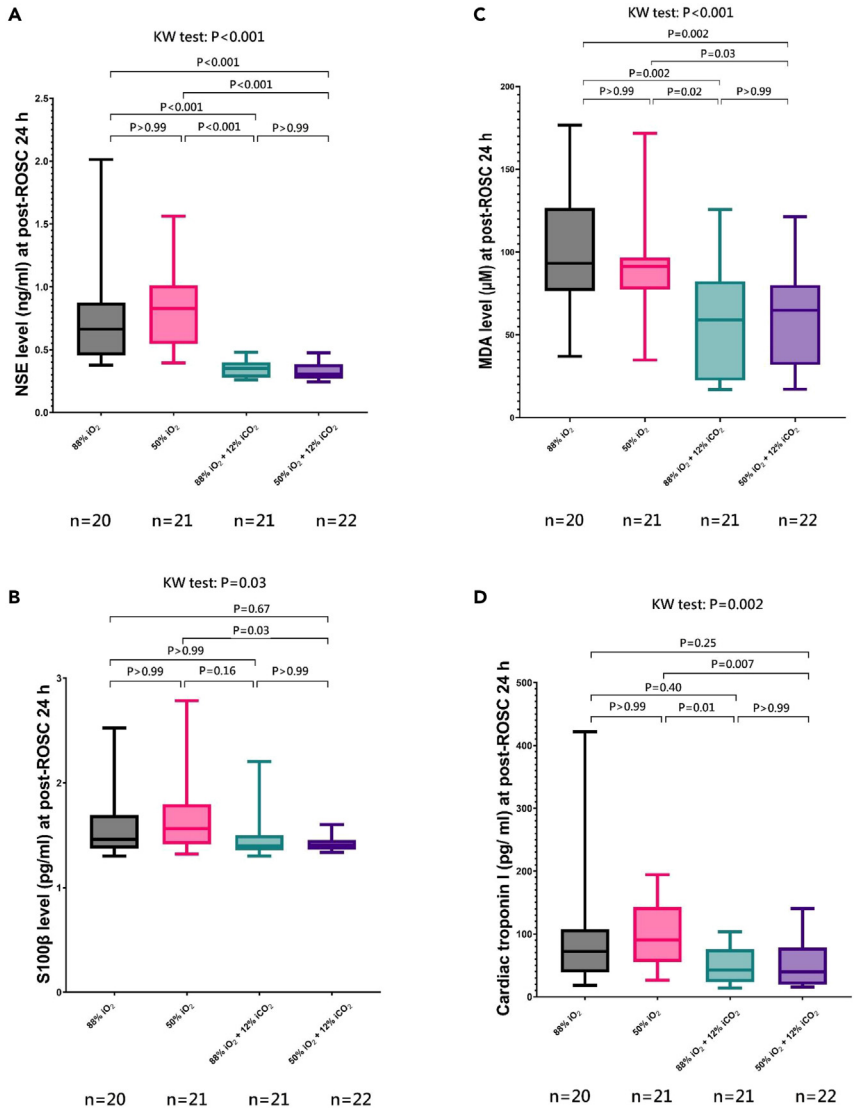

Figure 4. ELISA and TBARS assay at 24 h post-ROSC

(A) NSE levels.

(B) S100β levels.

(C) MDA: levels.

(D) Cardiac troponin I levels. Black: 50% iO_2 group; pink: 88% iO_2 group; green: 50% iO_2 + 12% iCO_2 group; purple: 88% iO_2 + 12% iCO_2 group. The neuronal injuries were evaluated using the NSE and S100β plasma concentrations. Oxidative injury was assessed using the MDA level in the hippocampus. Data are presented as a boxplot. Pairwise comparisons between experimental groups were performed using *post hoc* Dunn's test.

ELISA, enzyme-linked immunosorbent assay; iCO_2, inhaled carbon dioxide; iO_2, inhaled oxygen; KW: Kruskal-Wallis; MDA, malondialdehyde; NSE, neuron-specific enolase; ROSC, return of spontaneous circulation; TBARS, thiobarbituric acid reactive substance.

from 88% to 50% did not avoid post-ROSC cerebral hypoperfusion. In contrast, 12% iCO_2 significantly enhanced CBF, even if PaO_2 fell into the range of severe or moderate hyperoxia.

Figures 2C–2F show that, in the 88% and 50% iO_2 groups, $PbtO_2$ decreased gradually from the initial hyperemia stage, along with the declining CBF. Although PaO_2 in the 88% iO_2 group was nearly twice that in the 50% iO_2 group (Table 1), there were no significant differences in $PbtO_2$ between these two groups because the increase in PaO_2 only contributed to a small proportion of oxygen dissolved and transported by the CBF. In contrast, local tissue oxygenation is substantially determined by the regional blood flow,[15] and $PbtO_2$ may therefore be elevated by augmented CBF. Administration of 12% iCO_2 increased $PaCO_2$, causing dilation of cerebral arteries and arterioles and augmented CBF.[42] Despite various theories proposed for vasodilation induced by elevated $PaCO_2$, the primary mechanism seems to be

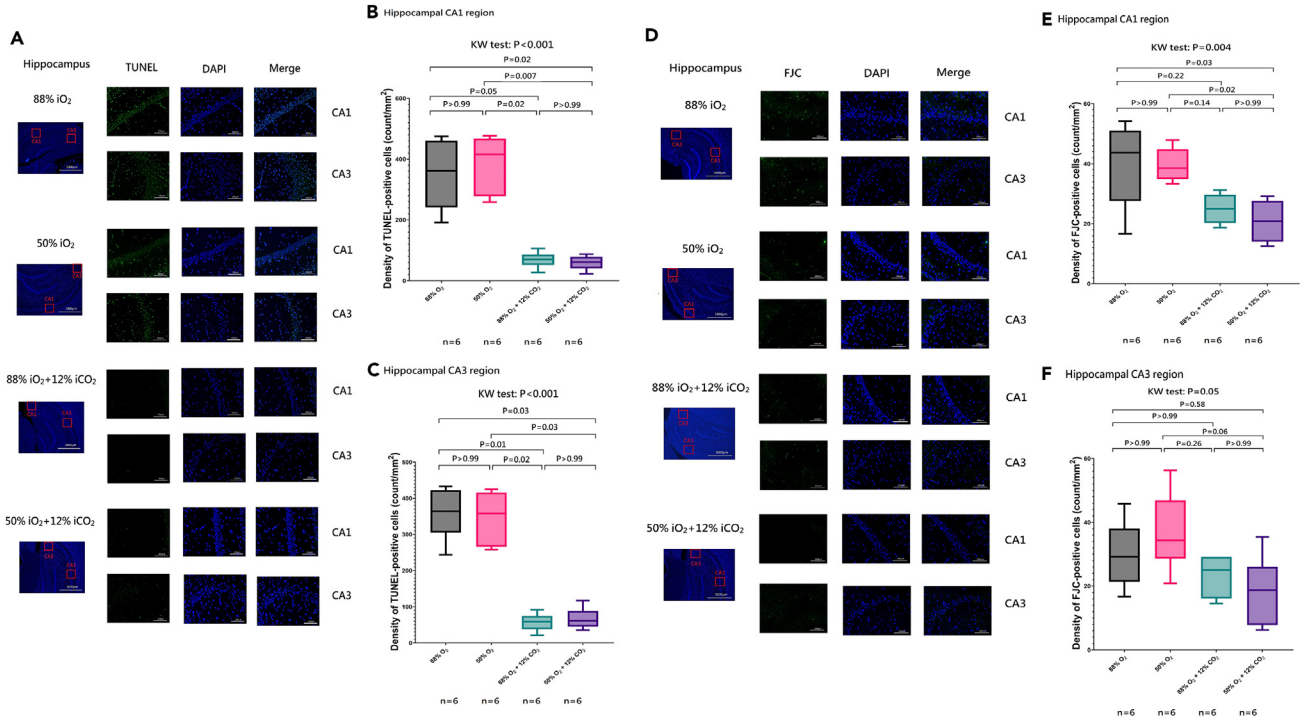

Figure 5. TUNEL assay and FJC staining at 24 h post-ROSC

(A) TUNEL assay of the hippocampus.

(B and C): Quantitative analysis of TUNEL assay for CA1 and CA3 regions, respectively.

(D) FJC staining of the hippocampus.

(E and F) Quantitative analysis of FJC staining for CA1 and CA3 regions, respectively. Black: 50% iO_2 group; pink: 88% iO_2 group; green: 50% iO_2 + 12% iCO_2 group; purple: 88% iO_2 + 12% iCO_2 group. Neuronal degeneration was evaluated by FJC staining. The hippocampal TUNEL-positive or FJC-positive cell density was used to quantify the level of apoptosis or neuronal degeneration, respectively. The coronal brain sections were subjected to TUNEL (green), FJC (green), and DAPI (bluish-violet) staining. Fluorescence microscopy was used to image hippocampal CA1 and CA3 regions at 50× magnification. Red rectangles indicate CA1 and CA3 regions selected for quantification. The scale bar represents 1000 μm. Samples were randomly selected for TUNEL assay or FJC staining. Data are presented as a boxplot. Pairwise comparisons between experimental groups were performed using *post hoc* Dunn's test.

DAPI, 4′,6-diamidino-2-phenylindole; FJC, Fluoro-Jade C; iCO_2, inhaled carbon dioxide; iO_2, inhaled oxygen; KW, Kruskal-Wallis; ROSC, return of spontaneous circulation; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

associated with the direct impact of extracellular hydrogen ions on the smooth muscle of blood vessels.[43] By augmenting CBF, 12% iCO_2 increased $PbtO_2$ more efficiently.

At 150 min post-ROSC, $PbtO_2$ was 8.33–12.87 mmHg and 35.05–42.03 mmHg in the groups without and with 12% iCO_2, respectively (Table 1). Normal $PbtO_2$ was suggested to be 23–35 mmHg but can vary depending on many factors, including measurement depth, and may be lower in deeper brain regions,[44,45] such as the hippocampal CA1 region monitored in our study. Hence, we calculated $\Delta PbtO_2$ to reduce the between-subject variability in absolute $PbtO_2$.[46] Sekhon et al.[47] noted that patients resuscitated from CA with a non-cardiac cause experienced a protracted period of critical cerebral hypoxia after ROSC, even when PaO_2 was controlled between 80 and 100 mmHg. Cerebral hypoxia was defined by Sekhon et al.[47] as $PbtO_2$ below 20 mmHg according to studies of traumatic brain injuries,[45,48] since there were no such definitions for post-ROSC patients. In the 88% and 50% iO_2 groups, the $\Delta PbtO_2$ data suggested that $PbtO_2$ at 150 min post-ROSC nearly returned to the baseline value (Table 1). If baseline $PbtO_2$ was considered normal, the $PbtO_2$ in the 88% and 50% iO_2 groups was above the normal $PbtO_2$ for most of the observation period, suggesting no post-ROSC brain hypoxia. In contrast, for the groups receiving 12% iCO_2, $PbtO_2$ substantially increased at 150 min post-ROSC, achieving a plateau significantly higher than the baseline.

It is possible that, even if $PbtO_2$ in the 88% and 50% iO_2 groups was above the baseline, the brain tissue might still suffer from hypoxic injuries. As the CBF at 150 min post-ROSC was just above half of the baseline (Table 1), $PbtO_2$ may be maintained by increased oxygen extraction from CBF to brain tissue. Gong et al.[7] demonstrated that the oxygen extraction ratio increased significantly after ROSC. Moreover, Hifumi et al.[49] noted an association of elevated lactate-to-pyruvate ratio (i.e., increased anaerobic metabolism) in brain tissue with poor neurological outcomes after CA. Therefore, 12% iCO_2 may elevate $PbtO_2$ to a new homeostatic state, maintaining the balance between oxygen delivery and consumption.

Clinical outcomes and pathological findings

There were no significant differences between the 88% and 50% iO_2 groups in the NFS and pathological findings, regardless of iCO_2 concentrations. Similarly, using an asphyxia-induced CA rat model, Lipinski et al.[50] indicated that 21% iO_2 during 60 min post-ROSC did not improve the neurological deficit or the histological injury, compared with 100% iO_2. The differences in post-ROSC CBF patterns and severity of brain damage between VF- and asphyxia-induced CA[6,51] may explain why reducing post-ROSC iO_2 concentration in our study did not demonstrate significant neuroprotective effects as in previous studies.[52]

In most clinical studies, PaO_2 >300 mmHg was associated with poor post-CA neurological recovery[32] or mortality.[26] Nevertheless, most of these studies were based on observations in intensive care units.[26,29,32] Our study suggested that the difference in adverse effects between 88% and 50% iO_2 administered for a short interval during the early post-ROSC period may not be as significant as the difference between high and low PaO_2 exposure during a later period for a prolonged duration. This result may be reassuring since, in clinical practice, patients may receive oxygen at a high concentration for 30 min to several hours during the early post-ROSC period[32,36,38,53] until they are transported to the hospital, where safe monitoring is possible. In the EXACT trial,[36] even if patients were targeted at SpO_2 between 98 and 100%, approximately 16.1% of them still experienced hypoxia (SpO_2 <90%) before admission to an intensive care unit, which was worrying since hypoxia may be more detrimental than hyperoxia.[54] In the early post-ROSC period, therapeutics other than adjusting iO_2 concentration may be needed to mitigate the injuries caused by hyperoxia.

Currently, the clinical evidence regarding the effects of $PaCO_2$ on post-ROSC neurological recovery is inconsistent.[35,55,56] Our results revealed that the iCO_2 could improve neurological outcomes (Figure 3) even when PaO_2 was above the physiological range and may potentially cause hyperoxic injuries. The administration of iCO_2 was associated with reduced NSE and MDA levels[57] (Figure 4) and lower TUNEL-positive and FJC-positive cell densities (Figure 5). No significant differences in caspase-3 activation or PARP cleavage were detected between groups with and without iCO_2 (Figure S5). As western blotting was performed using brain tissue harvested at 24 h post-CA, initial apoptotic phases may have been completed at this time. Administering iCO_2 may potentially cause severe acidosis and unfavorable clinical outcomes.[58] Although no iCO_2-associated adverse effects were observed in the current study, examining the effects of lower iCO2 concentrations against hyperoxic injuries in future studies would be worthwhile.

Finally, clinical studies indicated potential interactions between PaO_2 and $PaCO_2$, demonstrating that the combination of moderate hyperoxia and mild hypercapnia was associated with lower mortality[59] and better neurological outcomes[27] after CA. Similarly, our sensitivity analysis indicated an interaction between iO_2 and iCO_2, and the *post hoc* analysis suggested that the NFS was highest for the 50% iO_2+12% iCO_2 group. Therefore, 50% iO_2 and 12% iCO_2 might be the optimal combination for brain reoxygenation among all tested groups.

Conclusions

Facilitating brain reoxygenation may improve post-CA neurological outcomes. Reducing iO_2 concentration from 88 to 50% has little influence on post-ROSC CBF and $PbtO_2$. In contrast, regardless of iO_2 concentration, administering 12% iCO_2 could facilitate brain reoxygenation and neurological recovery.

Limitations of the study

The first limitation of this study is that it was performed using healthy animals. The impact of underlying disease on the recovery of CBF or $PbtO_2$ by iCO_2 is unknown. Thus, further studies on disease models are needed to clarify this issue. Second, only male animals were employed in the current experiment. Estrogen has been suspected to influence post-CA outcomes.[60] Therefore, most CA experiments were performed in male animals.[61,62] The generalizability to female subjects warrants further investigation. Third, neither the cerebral tissue oxygen extraction ratio nor the lactate-to-pyruvate ratio was measured, which limited our understanding of the mechanism by which increased CBF and $PbtO_2$ led to better neurological recovery. Fourth, the CPR time was numerically shorter in the 50% iO_2+12% iCO_2 group than in the 50% iO_2 group. Despite the absence of statistically significant between-group difference (Figure S2), it remained possible that the shorter CPR duration could partially explain the better outcomes in the 50% iO_2+12% iCO_2 group.[63] However, in our study, the rats were randomized in a block size of four to avoid the problem that the rats resuscitated in a temporally later period were resuscitated by investigators with more mature skills after repeated experiments. The Kaplan-Meier curves (Figure 3A) also do not show significant between-group differences in survival. Therefore, the numerical differences in the CPR time may occur by chance rather than systematic bias. Fifth, we used iCO_2 to achieve hypercapnia instead of hypoventilating the animals. This selection was made because the effects of iCO_2 on physiological parameters, including CBF and $PaCO_2$, were predictable and consistent according to our previous study results.[14] Nonetheless, using iCO_2 may not be readily feasible in clinical practice and needs more studies to support its use. Our results may be considered hypothesis generating. Further studies are warranted to examine if a less aggressive method, i.e., hypoventilation controlled by mechanical ventilators, could achieve similar outcomes.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.108476.

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AUTHOR CONTRIBUTIONS

C-H.W.: Conceptualization, Methodology, Validation, Resources, Formal analysis, Investigation, Data curation, Writing – original draft, Project administration; W-T.C.: Validation, Resources, Writing – review and editing, Project administration; C-H.H.: Resources, Writing – review and editing; M-S.T.: Writing – review and editing; C-C.W.: Investigation, Project administration; S-H.L.: Conceptualization, Methodology, Validation, Resources, Formal analysis, Writing – review and editing, Supervision; W-J.C.: Conceptualization, Methodology, Validation, Resources, Formal analysis, Writing – review and editing, Supervision.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Caspase-3 (D3R6Y) Rabbit mAb	Cell Signaling Technology	#14220; RRID:AB_2798429
PARP Antibody	Cell Signaling Technology	#9542; RRID:AB_2160739
Rat NSE(Neuron Specific Enolase) ELISA Kit	Elabscience Biotechnology	Cat.No.:E-EL-R0058
Rat S100B(S100 Calcium Binding Protein B) ELISA Kit	Elabscience Biotechnology	Cat.No.:E-EL-R0868
Rat Cardiac Troponin I ELISA Kit	Abcam	#ab246529
TBARS Assay Kit	Cayman Chemical	Item No. 10009055
DeadEnd™ Fluorometric TUNEL System	Promega	G3250
Software and algorithms		
ImageJ	National Institutes of Health	https://ImageJ.net/ij/index.html
Other		
FLUORO-JADE® C	EMD Millipore Corporation	Product Catalog Number:AG325-30MG, Lot # 3126966

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Wen-Jone Chen (wjchen1955@ntu.edu.tw).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

This research was approved by the Institutional Animal Care and Use Committee of the College of Medicine, National Taiwan University (reference number: 20180314) and performed following the Guide for the Care and Use of Laboratory Animals.[64] The analysis and results were conducted and reported according to Animal Research Reporting of *In Vivo* Experiments guidelines.[65] Animals were housed in a temperature-controlled environment (22°C) under a 12:12 h dark/light cycle and had free access to food and water.

Surgical preparation

An established asphyxia-induced CA rat model[8,14,24] was adopted in this study. Fourteen-week-old male Wistar rats, weighing approximately 450–500 gm, were employed for experiments. Before surgery, all animals were fasted for 12 h with free access to water. The animals were anesthetized using intraperitoneal pentobarbital (45 mg/kg). Then, tracheal intubation was performed with the insertion of a 14G venous catheter (Angiocath, Becton Dickinson, NJ, USA), including the following steps: (1) employ forceps to extract the tongue and introduce a laryngoscope blade into the oral cavity for observing the vocal cords; (2) introduce the venous catheter (with the needle replaced by a blunt stylet) through the vocal cords; (3) connect the catheter to the ventilator circuit and fasten it under the chin using a suture. Mechanical ventilation (R407, RWD Life Science, Hofheim, Germany) was initiated at a tidal volume of 0.6 mL/100 g body weight, a frequency of 100 breaths/min, positive end-expiratory pressure of 5 cmH_2O, and an iO_2 concentration of 21%. CBF and $PbtO_2$ levels were continuously monitored using OxyFlo Pro and OxyLite Pro (Oxford-Optronix, Oxford, UK) instruments, respectively, via a fiber sensor (NX-BF/OFT/E, Oxford-Optronix) inserted through a small cranial window over the left hemisphere. The insertion site was marked on the dura with the stereotaxic coordinate anteroposterior (−3.5 mm) and lateral (2.0 mm) from the bregma. The rats were then fixed to a customized stereotaxic

frame in the supine position. The fiber sensor was inserted 2 mm from the dura into the dorsal hippocampal CA1 region.[66] The right femoral artery and left jugular vein were cannulated to obtain arterial blood pressure and blood samples and administer medications, respectively. Electrocardiographic leads (SP844, MEMSCAP, Skoppum, Norway) were connected to a data acquisition system (Labchart 8 software, AD Instruments, Sydney, Australia) via their corresponding transducers and signal conditioners. Rectal temperature was maintained at 36.5°C–37.5°C by a homeothermic control pad (RightTemp, Kent Scientific, Torrington, CT, USA).

Asphyxia-induced CA and CPR

Figure 1 shows the experimental protocol. CA was induced by clamping the endotracheal tube and defined as mean arterial pressure (MAP) $\leq$ 20 mmHg. Neuromuscular blockers were not administered before asphyxia. Asphyxia-induced CA experiments could proceed with or without neuromuscular blockers.[67] In our experiments, the rats were fixed to a customized stereotaxic frame in the supine position. Because of the tight fixation to the frame, neuromuscular blockers were not needed to avoid equipment disconnection.[61,67] CPR was started 7 min after asphyxia, with one intravenous epinephrine (0.002 mg/100 g) dose, one sodium bicarbonate (1 mEq/kg) dose, and chest compressions. Chest compressions were performed manually by using two fingers to tap on the center of the sternum forcefully and sharply. The investigator's remaining hand would hold and stabilize the rib cage, ensuring proper chest recoil. The chest compression frequency was 200 beats/min, and the depth was approximately 1/3 the anteroposterior thorax diameter. No audiovisual feedback was used during CPR. Instead, the quality of chest compression was recognized by observing the pressure waves in the arterial catheter, as suggested by previous studies.[61] Manual chest compression was adjusted to maintain MAP >40 mmHg to ensure high-quality CPR. During CPR, uninterrupted chest compressions led to a gradual rise in MAP within 30–60 s. Typically, signs of spontaneous cardiac activity emerged as MAP approached the 40 to 50 mmHg range, which usually took about 45–75 s of CPR. Chest compressions would persist until spontaneous arterial pressure waves were seen and an electrocardiographic signal was detected. During CPR, ventilatory iO_2 concentration was 100%. ROSC was defined as MAP $\geq$40 mmHg for 10 min. Rats were randomly allocated to four study groups 15 min after ROSC. A computer-generated random number list with a block size of four was generated for the randomization. Randomized animals were ventilated with the following different combinations of iO_2 and iCO_2 concentrations for 150 min: 88% O_2+12% N_2 (88% iO_2 group), 50% O_2+50% N_2 (50% iO_2 group), 88% O_2+12% CO_2 (88% iO_2+12% iCO_2 group), and 50% O_2+12% CO_2+38% N_2 (50% iO_2+12% iCO_2 group). Previous studies[68] have shown that epinephrine administration during CPR produced microvascular alterations during the first-hour post-ROSC, including vasoconstriction, capillary stasis, and prolonged cortical transit time, which may confound our observations of the effects of iO_2 and iCO_2 on CBF and $PbtO_2$. Therefore, epinephrine infusion was not used after ROSC. During the intervention, anesthesia depth was monitored by observing the heart rate, MAP, and response to painful stimuli (pedal withdrawal reflex). If required, additional doses of pentobarbital (10 mg/kg) were administered intravenously every 30 min to maintain a surgical plane of anesthesia. In contrast, neuromuscular blocking agents were not administered during the post-ROSC period to prevent equipment disconnection because of the tight fixation to the frame. Also, gasping was not observed during the intervention period because of the respiratory depression caused by the pentobarbital. Therefore, there may be no gasping-related effects on lung functions and haemodynamics.

Outcome measures of survival and neurological recovery

After a 150 min intervention, the rats were returned to their cages after the wounds closed. At 24 h post-ROSC, rat survival status was recorded; neurological outcomes were assessed using rat NFS (Table S1)[69] by researchers blinded to the treatment allocations. Rats were then humanely euthanised using intraperitoneal pentobarbital (45 mg/kg), followed by exsanguination.[70] After sacrifice, blood samples were collected from the right atrium; the right hippocampus was separated, cryofixed in liquid nitrogen, and stored at −80°C; the left harvested hippocampus was fixed in 4% formaldehyde in 0.1 M phosphate buffer.

METHOD DETAILS

Enzyme-linked immunosorbent assay (ELISA)

ELISA kits of NSE (#E-EL-R0058; Elabscience Biotechnology, Houston, TX), S100β (#E-EL-R0868; Elabscience Biotechnology), and cardiac troponin I (#ab246529; Abcam, Cambridge, UK) were used to determine their plasma levels in association with brain and heart injuries based on manufacturers instructions.

Thiobarbituric acid reactive substance (TBARS) assay

The specimens of the right hippocampus were homogenized in RIPA buffer with protease and phosphatase inhibitors (#78441; Thermo Fisher Scientific, Waltham, MA). After centrifugation, the resulting supernatant was analyzed with the TBARS assay kit (#10009055; Cayman Chemical, Ann Arbor, MI) to measure the concentration of MDA, a lipid peroxidation product.

TUNEL assay

To evaluate apoptosis, the specimens of the left hippocampus were assayed using the DeadEnd Fluorometric TUNEL System (G3250, Promega, Madison, WI) according to the manufacturer's instructions. TUNEL was performed on 4-μm-thick paraffin-embedded coronal sections. The cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). The apoptotic cells were examined by fluorescence microscopy (EVOS FL Auto Imaging System, Thermo Fisher Scientific, Waltham, MA, USA). For quantitative comparisons, viewing fields were randomly selected

in hippocampal CA1 and CA3 regions for each sample.[71] Each field was photographed under microscopy at a 510/42 nm wavelength (green TUNEL) and a 447/60 nm wavelength (bluish-violet DAPI). PhotoImpact X3 (Corel, Ottawa, Canada) was used to merge the two images for the final counting analysis with ImageJ software (Version 1.52a, National Institutes of Health, Bethesda, MD, USA). TUNEL-positive cell densities (count/mm^2) in the regions mentioned above were measured at 50× magnification, with an area of each microscopic field of approximately 0.48 mm^2.

FJC staining

To assess degenerating neurons, the specimens of the left hippocampus were stained with FJC (#AG325-30MG; EMD Millipore Corporation, Temecula, CA) per the manufacturer's instructions. Nuclei were stained with DAPI. The method of quantifying FJC-positive cells was the same as that using the TUNEL assay described above.

WB analysis

The specimens of the right hippocampus were used for WB. The primary antibodies used were for caspase-3 (#14220; Cell Signaling Technology, Danvers, MA) and PARP (#9542; Cell Signaling Technology), while b-actin (HRP-60008; Proteintech Group, Rosemont, IL) was used as a loading control.

QUANTIFICATION AND STATISTICAL ANALYSIS

The primary outcome of this study was NFS, which was used for *a priori* sample size calculation. For between-group comparisons, 17 animals/group were shown to be required to demonstrate a mean difference of 1.5 in NFS, with a power of 80% at the 5% level and a standard deviation of 1.5 (MedCalc, version 20.114; MedCalc Software, Ostend, Belgium). If the 24 h survival rate was assumed to be 70%,[8,24] the required size of each group was calculated to be 24 to compensate for losses. CBF was expressed as the proportion of the baseline (%baseline), and $\Delta PbtO_2$ was computed based on $PbtO_2$ at a particular time point minus the baseline.[46] Categorical data were expressed as count and proportion and compared using the chi-squared test. Continuous data were expressed as the median and interquartile range (IQR) and compared using the Kruskal-Wallis test. *Post hoc* Dunn's test was used for pairwise comparisons between each experimental group. Time-based measurements were compared using two-way repeated-measures ANOVA. Survival curves were created by the Kaplan-Meier method and compared by the log rank test. In sensitivity analysis, two-way ANOVA was used to examine the interaction between iO_2 and iCO_2 for NFS, and Tukey's test was used for *post hoc* pairwise comparisons. A two-tailed $p < 0.05$ was considered statistically significant. All statistical tests were performed in GraphPad Prism Version 9.5.0 (GraphPad Software, La Jolla, CA).

ADDITIONAL RESOURCES

None.