Emulsified isoflurane postconditioning improves survival and neurological outcomes in a rat model of cardiac arrest

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Abstract. Emulsified isoflurane (EIso) has a protective effect against ischemia/reperfusion (I/R) injury in animal models. However, the protective effects of EIso on global cerebral I/R injury remain unclear. The present study aimed to investigate whether EIso postconditioning was able to improve survival and neurological outcomes in a rat model of cardiac arrest (CA). Rats were randomly divided into five groups, namely the control, EIso-2ml, EIso-4ml, isoflurane (Iso) and emulsion (E) groups. All rats were resuscitated by a standardized method following 6 min of asphyxia. Furthermore, all interventions were administered immediately following the return of spontaneous circulation (ROSC). The animal survival was recorded daily, and evaluations of behavioral and brain morphology were assessed at 1 and 7 days after ROSC. The results showed that EIso treatment increased the survival rate 7 days after ROSC, with a 41.7% 7-day survival in the EIso-2ml group, 66.7% in the EIso-4ml group and 50% in the Iso group compared with 33.3% survival in the control and E groups. Moreover, the neural deficit score and memory function were improved in the EIso-4ml group, and this treatment also ameliorated brain hippocampal cell injury and apoptosis. In addition, a better brain protective effect was observed in the EIso-4ml group compared with the EIso-2ml, Iso and E groups. In summary, the data of the present study suggest that EIso postconditioning improved the survival and neurological outcomes following CA in a dose-dependent manner.

Introduction

As one of the most serious complications following cardiac arrest (CA), global cerebral ischemia/reperfusion (I/R) injury directly leads to serious neurological dysfunction and mortality. It has been reported that <5% of individuals with an out-of-hospital CA are able to survive, and only 25% of patients survive the subsequent hospitalization following initial resuscitation (1-4). Therefore, it is necessary to develop novel therapeutic options in order to reduce cerebral I/R injury in patients experiencing CA.

Ischemic postconditioning was first described by Zhao *et al* in 2003 (5), and consists of short episodes of ischemia after a prolonged cardiac ischemia to reduce cardiac infarct size. Previous results have also confirmed the neuroprotection effect of ischemic postconditioning (6). However, brain ischemic postconditioning may be difficult to apply in clinical practice due to potential lesions of brain vessels and tissues. Compared with ischemic postconditioning, inhaled anesthetic postconditioning is relatively easy to administer and carries low risk. It has been shown that postconditioning by the administration of inhaled anesthetics, including isoflurane (Iso), sevoflurane and xenon, can protect against cerebral I/R injury both *in vitro* and *in vivo* (7-14). The mechanisms responsible for anesthetic postconditioning are still unclear, but it is evident that anesthetic postconditioning and ischemic postconditioning have similarities, such as regulating pro-survival signaling downstream, activating the phosphoinositide 3-kinase/Akt pathway, attenuating mitochondrial damage and inflammation reaction, and reducing oxidative stress and apoptosis (11,15). However, the requirement for a vaporizer and related equipment limits the use of anesthetic postconditioning in pre-hospital resuscitation. A new type of anesthetic, emulsified isoflurane (EIso), can offset these limitations. As a recently developed formulation (a combination of emulsion and Iso), EIso is suitable for intravenous administration. Large-animal experiments indicated that EIso provides more rapid induction and recovery compared with propofol, and presents a notable hemodynamic stability (16). A few animal studies have shown that EIso possesses protective effects on ischemic heart, liver and lung (17-20). However, studies on EIso for global cerebral I/R injury are very limited.

Recently, a phase I clinical trial of EIso has been completed and the results have shown that EIso possesses a strong anesthetic potency and can be safely used in humans (21). Therefore, it was hypothesized that EIso postconditioning, administered following the return of spontaneous circulation (ROSC), could provide cerebral protection (22). The present study aimed to

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evaluate whether EIso postconditioning improved the survival and neurological outcomes in a rat model of CA.

Materials and methods

Animals. A total of 73 healthy male adult Sprague-Dawley rats (250-350 g, ~2 months old, provided by the Center of Experimental Animals at Sichuan University, Chengdu, China) were used in the present study, and the animal experiment protocol was approved by the Institutional Animal Experimental Ethics Committee of West China Hospital, Sichuan University (approval number: 20120112B). The animals were maintained under a 12:12 h light/dark cycle in a temperature- (20-25˚C) and humidity-conditioned (60±5%) environment, and rats were permitted free access to food and water. All animals were handled in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all efforts were made to minimize suffering during experiment under appropriate anesthesia and practice of appropriate animal handling skills. EIso was prepared in our laboratory according to a well-established protocol (18). The concentration of EIso was then determined and confirmed by gas chromatography (Aligent 4890 D; Tegent Technology Ltd., Shanghai, China) at the beginning of the experiments.

Animal preparation. Under general anesthesia (10% chloral hydrate, 300 mg/kg, Chengdu Kelong Chemical Co., Ltd., Chengdu, China), the rats were orotracheally intubated with a 16-G cannula (B. Braun Melsungen AG, Melsungen, Germany) and mechanically ventilated using a rodent ventilator (HX-300S; Chengdu Taimeng Technology Co., Ltd., China) with room air at a tidal volume of 10 ml/kg. The respiratory rate was set at a frequency of 60 breaths/min to maintain normocapnia. Furthermore, the rectal temperature was kept at 37±0.5˚C throughout the experiment with the aid of a heating lamp. The femoral artery cannulation was established with a 24-G catheter for blood pressure monitoring and blood sampling. Another 22-G catheter was inserted into the left femoral vein for drug administration. Arterial blood was collected for blood gas analysis (ABL800; Radiometer Inc., Copenhagen, Denmark) at three time points as follows: Baseline, and 30 and 60 min after ROSC. The mean arterial pressure (MAP) and electrocardiogram were continuously monitored using a physiological recorder (Biolap420E; Chengdu Taimeng Technology Co., Ltd.).

CA and resuscitation. The anesthetized rats were paralyzed with succinylcholine [0.5 mg/kg intravenously (i.v.); Shanghai Xudong Haipu Pharmaceutical Co., Ltd., Shanghai, China]. Subsequently, asphyxia was induced by stopping the ventilator and clamping the tracheal tube at the end of exhalation. CA was defined as a systolic blood pressure ≤25 mmHg. Resuscitation was achieved after 6 min of asphyxia. Briefly, rats were mechanically ventilated with 100% oxygen at a tidal volume of 10 ml/kg, the respiratory rate was set at a frequency of 80 breaths/min, and the thoracic compression was maintained at a rate of 200 compressions/min. Adrenaline (0.02 mg/kg i.v.; Grand Pharmaceutical Co., Ltd., Wuhan, China) and 5% $NaHCO_3$ (1 mmol/kg i.v.; Huiyinbi Group Jiangxi Dongya Pharmaceutical Co., Ltd., Jiangxi, China) were simultaneously administered by thoracic compression. ROSC was defined as an increase of MAP of >50 mmHg for >10 min (23).

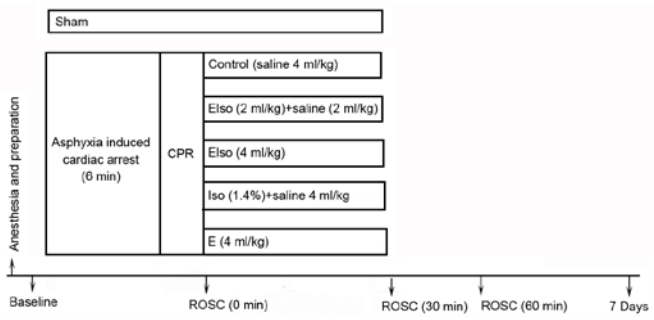

Figure 1. Experimental protocols. The animals were divided into five groups after successful ROSC, namely the control, EIso-2ml, EIso-4ml, Iso and E groups as shown. CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; EIso, emulsified isoflurane; Iso, isoflurane; E, emulsion.

After a successful resuscitation, mechanical ventilation was maintained until spontaneous respiration was well restored. At 1 h after ROSC, the catheters were withdrawn, vessels were ligated, and the wounds were closed. Following extubation, the rats were placed in a chamber with 50% O_2 for 30 min, and then room air was provided for 30 min. Finally, rats were returned to their cages.

Experimental design. After ROSC, 60 rats were randomly and evenly assigned into five groups (12 rats in each group), and a further 4 rats were included in the sham group (without CA and CPR) for brain morphology analysis (Fig. 1). All interventions were administered immediately among post-CA rats after ROSC and maintained for 30 min. The control group received an intravenous infusion of saline (4 ml/kg). The EIso-2ml group received an intravenous infusion of EIso (2 ml/kg) and saline (2 ml/kg). The EIso-4ml group received an intravenous infusion of EIso (4 ml/kg). The Iso group inhaled 1.4% Iso and received an intravenous infusion of saline (4 ml/kg) and finally, the emulsion (E) group received an intravenous infusion of emulsion (4 ml/kg).

Survival condition and neurological function evaluations. Post-CA rats were monitored for 7 days, and the survival rate was recorded daily. All evaluations of the neurological functions were blindly performed by an independent investigator 1 day prior to the operation, and 1 and 7 days after ROSC.

Neural deficit score (NDS). Neurological functions were assessed with an established NDS (24), which evaluates general behavior and cranial function, sensory and motor function and coordination. A normal rat has an NDS of 500, and 0 represents mortality.

Novel object recognition test. This test was performed based on the spontaneous tendency of a rat to explore a novel object, and it was conducted as previously described by Bevins and Besheer (25). Briefly, 1 day prior to the test, each rat was allowed to stay in the test apparatus for 20 min in order to

Table I. Physiological variables before and after restoration of spontaneous circulation.

Variable	Control	EIso-2ml	EIso-4ml	Iso	E
Baseline					
Body weight (g)	272±31	280±14	272±23	289±20	289±26
MAP (mmHg)	97±17	93±18	98±13	90±19	98±15
Body temperature (˚C)	36.9±0.5	37.2±0.5	37.0±0.4	36.8±0.3	37.0±0.5
pH	7.35±0.03	7.34±0.02	7.34±0.03	7.36±0.04	7.36±0.02
$PaCO_2$ (mmHg)	35±5	35±4	34±6	32±4	36±4
PaO_2 (mmHg)	192±55	239±80	241±98	195±68	196±89
SaO_2 (%)	96.4±2.1	96.6±1.5	97.0±0.7	96.4±1.7	96.2±1.4
HCO_3^- (mmol/l)	19.7±1.5	19.2±.4	19.0±2.0	19.2±2.2	20.5±1.2
Base excess (mmol/l)	-6.0±2.1	-6.6±1.9	-6.9±2.8	-6.8±2.5	-5.0±1.8
Hemoglobin (g/dl)	14.1±1.2	13.4±1.6	12.8±2.4	13.2±2.0	14.3±0.9
Potassium (mmo/l)	3.8±0.4	3.9±0.5	3.5±0.7	3.6±0.6	3.8±0.6
Lactic acid (mmol/l)	1.1±0.4	1.1±0.4	0.9±0.3	1.3±0.8	1.1±0.3
At 30 min after ROSC					
MAP (mmHg)	70±14	66±11	71±17	65±14	79±10
pH	7.29±0.09	7.22±0.07	7.27±0.07	7.27±0.10	7.28±0.06
$PaCO_2$ (mmHg)	34±11	36±6	35±7	34±14	38±11
PaO_2 (mmHg)	136±24	120±24	110±15	120±19	124±27
SaO_2 (%)	93.2±3.0	90.6±4.2	90.7±2.8	91.3±3.9	91.2±3.0
HCO_3^- (mmol/l)	16.3±2.0	15.8±2.5	16.2±2.1	15.8±2.1	17.2±1.2
Base excess (mmol/l)	-10.3±2.6	-10.7±3.2	-10.2±2.9	-10.9±3.1	-8.7±2.1
Hemoglobin (g/dl)	15.1±2.0	13.2±1.0	12.7±1.9	13.0±2.2	14.1±2.7
Potassium (mmol/l)	4.7±1	4.0±0.9	4.2±0.8	4.5±1	4.6±0.9
Lactic acid (mmol/l)	3.0±0.9	2.7±1.7	2.5±0.9	3.0±0.4	2.4±0.8
At 60 min after ROSC					
MAP (mmHg)	71±16	71±15	76±21	78±20	81±8
pH	7.29±0.06	7.26±0.06	7.24±0.07	7.28±0.09	7.29±0.04
$PaCO_2$ (mmHg)	42±7	43±8	46±7	40±10	42±9
PaO_2 (mmHg)	160±54	142±36	139±28	149±50	157±50
SaO_2 (%)	94.8±2.5	92.7±2.6	92.3±3.1	92.8±3.8	94.1±2.2
HCO_3^- (mmol/l)	19.2±2.1	17.8±1.8	18.8±2.5	18.0±2.2	18.9±1.6
Base excess (mmol/l)	-6.0±2.7	-7.6±2.5	-5.8±3.5	-7.6±2.7	-6.4±2.6
Hemoglobin (g/dl)	15.7±1.4	13.7±1.6	14.6±2.0	14.3±1.7	14.9±1.3
Potassium (mmol/l)	5.0±0.7	4.8±1.0	5.1±1.0	4.7±1.0	4.3±0.9
Lactic acid (mmol/l)	2.0±0.8	2.3±1.2	2.0±0.6	2.7±1.2	1.9±0. 4

All values are the mean ± standard deviation. No significant differences were observed among the groups. EIso, emulsified isoflurane; Iso, isoflurane; E, emulsion; MAP, mean arterial pressure; ROSC, return of spontaneous circulation; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; SaO_2, oxygen saturation.

become familiar with the environment. At the beginning of the test, two identical objects (sample objects) were positioned in the back left and right corners of the apparatus. The rat was placed at the mid-point of the wall opposite the objects, with its body parallel to the sidewalls, and its nose pointed away from the objects. Subsequently, the rat was given 10 min to freely explore the objects in the apparatus, and then it was placed back to its home cage. After 1 h, one of the sample objects and a novel object were replaced in the apparatus, and the rat was placed in exactly the same manner as described above. The movement of the rat was monitored for 5 min, and the time that the rat interacted with both objects was recorded. The recognition index (RI=novel object interaction time/total object interaction time) was determined for later analysis, where RI reflects the novel object discrimination capability of rats and a higher RI demonstrates a better memory condition.

Contexual fear conditioning. Fear conditioning represents a form of associative learning that has been well used in numerous species, including rats (26,27). Briefly, 1 day prior the operation, the subject rat was placed in a chamber and given 120 sec for free exploration. Next, a mild foot shock (0.8 mA for 1 sec) was

administered, and such a procedure was repeated five times with intervals of 120 sec. At day 1 and 7 after ROSC, the rat was placed into the same training chamber for 5 min, during which the presence of freezing response of the rat (absence of movement except for respiration) was observed and the freezing time was recorded for later analysis.

Brain morphology evaluations. At 7 days after ROSC, the rats were sacrificed and 10-μm paraffin-embedded coronal sections were prepared at the hippocampal level (approximately at bregma -3.0 mm). Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining was adopted to detect DNA fragmentation as previously described (28). The hippocampal CA-1 sector was thoroughly analyzed at a magnification of x400 by counting all TUNEL-positive cells (CAST system, Revision 0.9.5; Olympus, Ballerup, Denmark). In order to evaluate viable neurons of the hippocampal CA-1 sector, the same method was used on Nissl-stained 10-μm sections at the same level of the hippocampus. These examinations were blindly performed by a pathologist.

Statistical analysis. Data are expressed as the mean ± standard deviation. The homogeneity of variance was evaluated using Levene's test. Physiological variables and viable neuron counts were analyzed by one-way analysis of variance with a Bonferroni post hoc test between multiple experimental groups. Furthermore, the survival rate was compared using Fisher's exact test, and the 7-day survival condition was compared using Kaplan-Meier analysis and log-rank test. Statistical analysis was performed using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Physiological variables. The results shown in Table I reveal that there were no significant differences among the five groups in terms of physiological variables at baseline, 30 and 60 min after ROSC. In addition, as shown in Table II, no significant differences were observed in resuscitation-associated variables with regard to the asphyxia time (time from ventilation termination to CA), CA time [time from CA to initiation of cardiopulmonary resuscitation (CPR)] and ROSC time (time from initiation of CPR to ROSC).

Survival conditions. A total of 73 rats were included in the present study, among which 4 rats were included in the sham group for brain morphology analysis, 7 rats failed to achieve ROSC and 2 rats died due to blood loss caused by femoral artery cannulation failure. The survival rate 1 day after ROSC in the EIso-4ml group was significantly higher than in the control group (100 vs. 58.3%, $P<0.05$). Furthermore, the survival rate 7 days after ROSC was 33.3% in the control and E groups, and 41.7, 66.7 and 50% in the EIso-2ml, EIso-4ml ($P<0.05$ vs. control group) and Iso groups, respectively (Fig. 2).

NDS. At 1 day after ROSC, the EIso-4ml and Iso groups showed higher NDS values compared with the control group. At 7 days after ROSC, NDS values were still higher in the EIso-4ml and Iso groups compared with the control group, and the EIso-4ml group also exhibited a higher NDS than the EIso-2ml and E groups (Fig. 3A and B).

Table II. Times of different procedures during cardiac arrest and cardiopulmonary resuscitation.

Procedure	Control	EIso-2ml	EIso-4ml	Iso	E
Asphyxia (sec)	193±35	197±20	196±38	197±20	192±14
CA (sec)	167±35	163±20	164±38	163±20	168±14
ROSC (sec)	68±23	65±21	69±21	68±21	66±20

All values are the mean ± standard deviation. No differences were observed among the groups. Asphyxia time, time from ventilation termination to CA; CA time, time from CA to initiation of CPR; ROSC time, time from initiation of CPR to ROSC. CA, cardiac arrest; ROSC, return of spontaneous circulation; EIso, emulsified isoflurane; Iso, isoflurane; E, emulsion.

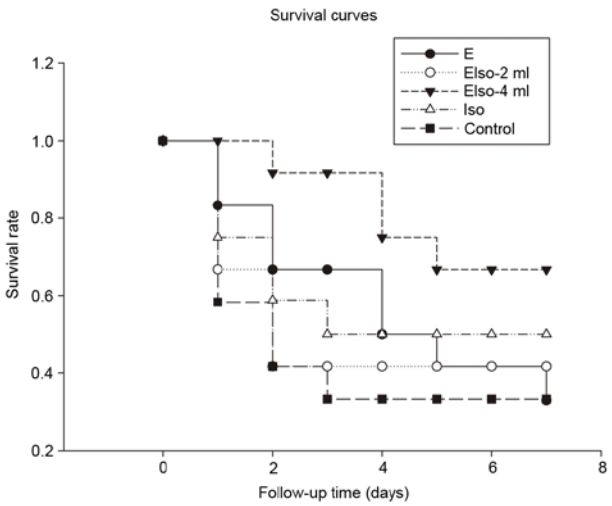

Figure 2. Kaplan-Meier survival curves of five groups. The EIso-4ml group had a higher survival rate than the control group (P=0.038). EIso, emulsified isoflurane; Iso, isoflurane; E, emulsion.

Novel object recognition test. At 1 day after ROSC, the EIso-4ml group showed a greater RI than the other four groups. At 7 days after ROSC, the RIs in the EIso-2ml and EIso-4ml groups were both higher compared with that of the control group (Fig. 3C and D).

Contextual fear conditioning. At 1 day after ROSC, the freezing times in the EIso-4ml and Iso groups were greater than that in the control group. At 7 days after ROSC, the freezing time in the EIso-4ml group was greater than that in the control, EIso-2ml and E groups (Fig. 3E and F).

Brain morphology evaluations. Compared with the sham group (n=4), the viable neurons in the other five groups were significantly decreased 7 days after ROSC. The number of viable neurons in the hippocampal CA-1 region was significantly preserved in the EIso-4ml group compared with the control, EIso-2ml and E groups. The number of TUNEL-positive

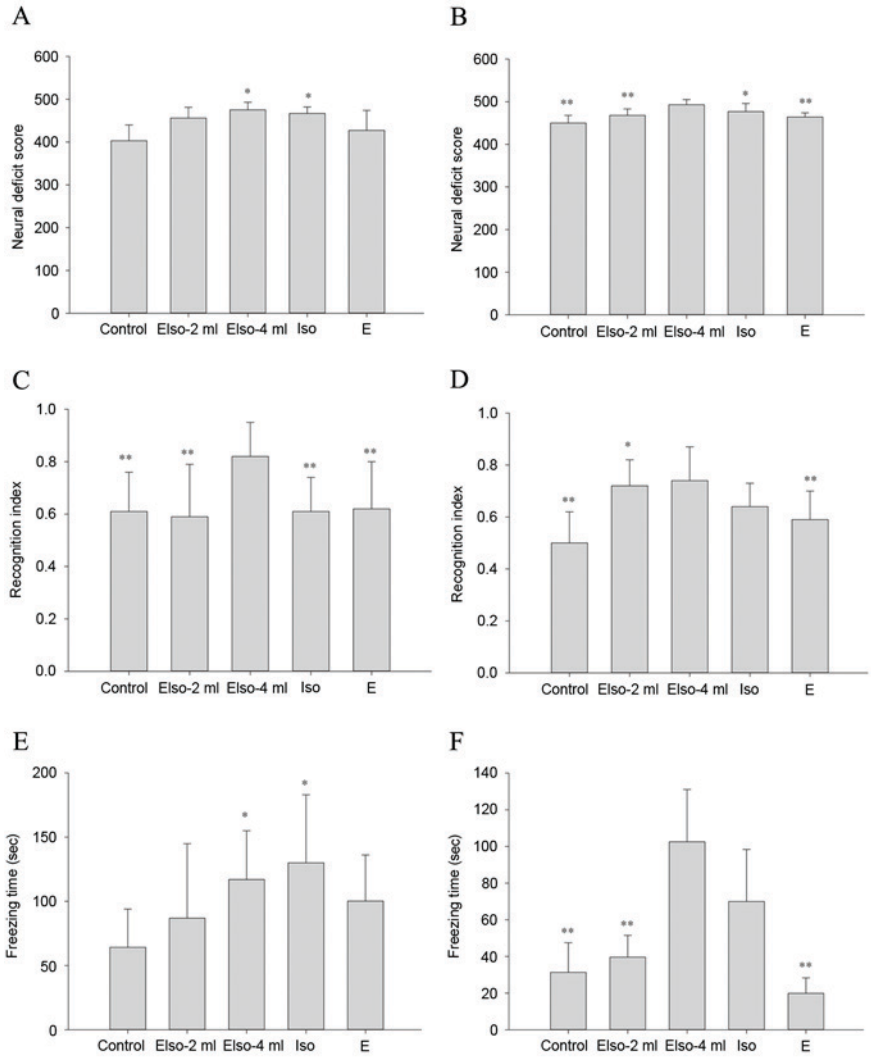

Figure 3. Behavioral evaluations at 1 day and 7 days after the return of spontaneous circulation. Neural deficit score at (A) 1 day and (B) 7 days; recognition index in the novel object recognition test at (C) 1 day and (D) 7 days; and freezing time in the contextual fear conditioning test at (E) 1 day and (F) 7 days. *P<0.05 vs. the control group. **P<0.05 vs. the EIso-4ml group. EIso, emulsified isoflurane; Iso, isoflurane; E, emulsion.

cells in the EIso-4ml group was less than those in the control, EIso-2ml and E groups (Fig. 4).

Discussion

The present study showed that EIso postconditioning had a beneficial effect on cerebral I/R injury. Additionally, EIso also showed a dose-dependent response for its cerebroprotective effects. Intravenous administration of volatile anesthetics may have some advantages compared with the inhaled mode of delivery, such as more rapid induction and recovery as well as ease of pre-hospital administration (16). Organ protection has previously been achieved by EIso preconditioning in different animal models, with protection of the heart, liver, kidney and lungs being observed (19,29,30). Anesthetic postconditioning has been shown to be as effective as preconditioning; Hu *et al* (31) have demonstrated that postconditioning with EIso at the start of reperfusion is capable of producing myocardial protection against I/R injury. A previous study of EIso has shown that a lower dose of Iso (~80% less for anesthetic induction and 20% less for maintenance) is required to obtain comparable anesthetic and organ-protective effects (32). In the present study, EIso postconditioning profoundly improved the survival of rats, and neurological outcomes were also enhanced at 1 and 7 days after ROSC. The results confirmed and extended previous findings. To the best of our knowledge, the present study for the first time indicated that administration of EIso following ROSC improved the survival and neurological outcomes in a rat model of CA.

Although the EIso-2ml and Iso groups showed improved NDS values, memory function and survival conditions, no significant protective effect on brain morphology was detected. Previous studies demonstrated that EIso (2 ml/kg) is useful for preventing heart injury in rats, and apoptotic cardiomyocytes are significantly reduced in EIso-treated rats compared with the control group (18,31). Fang *et al* (14) reported that cerebral infarct ratios were significantly decreased in rats post-conditioned with 1.4% Iso for 30 min using a middle cerebral artery occlusion (MCAO) model. Another study demonstrated that postconditioning of myocardial infarction with EIso (2 ml/kg) is as effective as 1 minimal alveolar concentration (MAC) of Iso in rats (32). Since the present study is our first of EIso in a

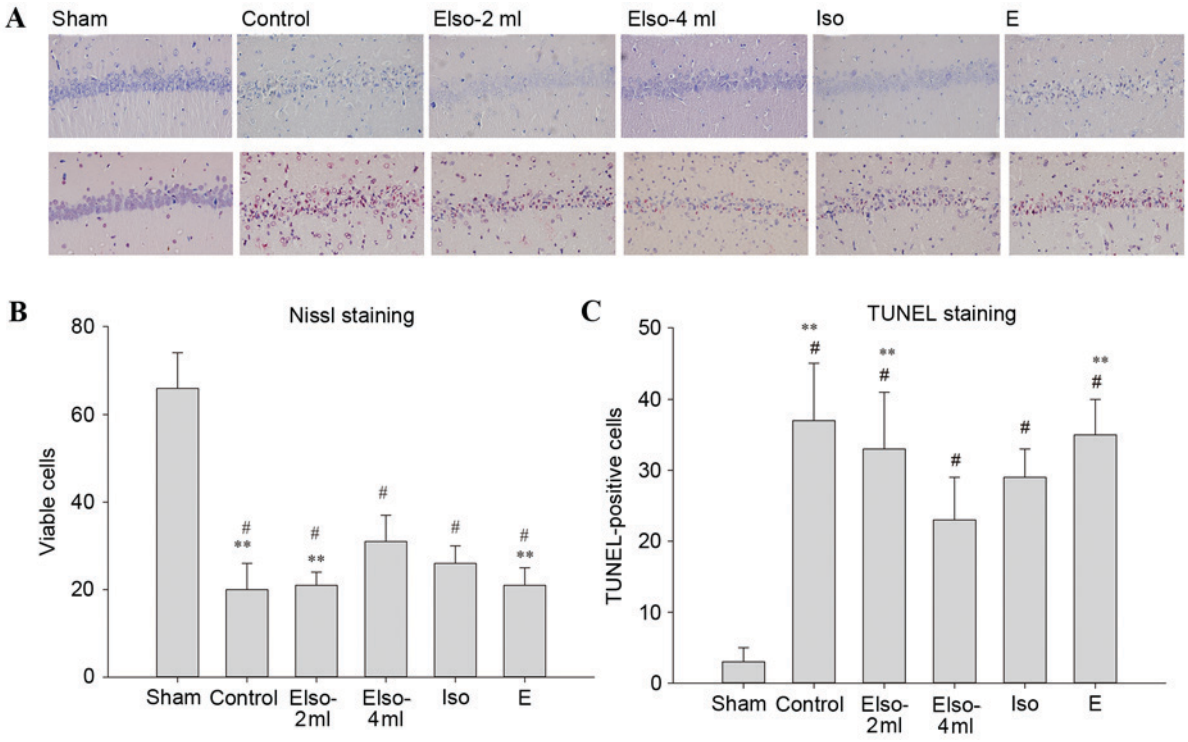

Figure 4. Nissl and TUNEL staining of the hippocampal CA-1 zone 7 days after the return of spontaneous circulation. (A) The upper images show Nissl staining and the lower images show TUNEL staining. Magnification, x400. (B) Viable cells detected by Nissl staining and (C) TUNEL-positive cells in the CA-1 hippocampal sector of the sham group (n=4) and the experimental groups (n=4-6) 7 days after restoration of spontaneous circulation. $^{\#}$P<0.05 vs. the sham group; **P<0.05 vs. the EIso-4ml group. EIso, emulsified isoflurane; Iso, isoflurane; E, emulsion; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

rat model of CA, EIso (2 ml/kg) and 1.4% Iso (corresponding to 1 MAC of Iso in rats) were selected as interventions based on the previous studies. The results of the present study showed that EIso (2 ml/kg) and 1.4% Iso did not manifest significant protective effects on cerebral I/R injury following CA. Possible explanations for these results were the differences in the target organ and cerebral ischemic model. The greatest proportion of the post-CA mortality and morbidity is caused by global ischemic brain damage, and brain neurons are more sensitive to ischemic insult compared with cardiomyocytes (33). Furthermore, EIso (2 ml/kg) may have useful effects on ischemic cardiomyocytes, but did not show significant benefits on an ischemic brain. In addition, ischemic injury in the CA model was more serious and diffused compared with the occlusion of left anterior descending coronary artery and MCAO models. Therefore, the discordance between a previous MCAO model and the present study is possible, and further studies should be performed to elaborate on this observation.

Due to the lack of knowledge regarding the appropriate intravenous dosage of EIso for cerebral I/R injury protection, the references used for guidance were based on EIso in myocardial protection. Chiari *et al* (30) demonstrated that intravenous infusion of 6.9% EIso (3.5 ml/kg/h) for 30 min has a protective effect on rabbit models of myocardial infarction. In addition, Rao *et al* (17) revealed that continuous infusion of 8% EIso (2-3 ml/kg) exerts infarct-limiting effects on rabbits. Furthermore, EIso (2 and 4 ml/kg) have better effects on an ischemic heart in rats compared with EIso (1 ml/kg) (34). Therefore, the dose-dependence of EIso indicates that a higher dose of EIso may have better protective effects. Besides EIso (2 ml/kg) and 1.4% Iso, EIso (4 ml/kg) was additionally selected to evaluate the dose-effect on cerebral ischemia following CA. The results revealed that EIso (2 ml/kg) and 1.4% Iso indeed exhibited a tendency to improve survival conditions. However, EIso (4 ml/kg) had a better effect in terms of both the survival rate and neurological outcomes compared with EIso (2 ml/kg) and Iso.

Although it is necessary to further explore the dose-effect relationship between EIso and cerebral protection, it may be concluded that EIso (4 ml/kg) had a better protective effect than EIso (2 ml/kg) and 1.4% Iso on cerebral I/R injury following CA in rats. Furthermore, previous studies have demonstrated that ischemic brain injury is a process characterized by progressive neuronal loss for at least 7-14 days after ischemic insults in rodents (35,36). The results of the present study highly support this, demonstrating that EIso (4 ml/kg) significantly reduced apoptotic cells and protected living neurons 7 days after ROSC.

More importantly, emulsion is critical in this protective process. There is evidence that intravenous emulsion therapy has a positive effect on hemodynamics (37,38), and it protects the heart by decreasing apoptosis (39,40). In the experiments of the present study, MAP at 30 and 60 min after ROSC in the E group was the highest among five groups, and memory function and survival rate at 1 day after ROSC in the E group were better compared with the control group, although these differences were not statistically significant. Therefore, it was possible that emulsion reduced hemodynamic changes and improved the blood supply and oxygen of the brain following CPR, which consequently improved the survival rate and neurological outcomes. As a combination of emulsion and Iso,

the protective effect of EIso may be associated with the emulsion. However, this speculation cannot be confirmed until further mechanistic studies are conducted.

Nevertheless, the present study has certain limitations. Firstly, although two different Elso dosages were investigated, it is not possible to conclude an appropriate dosage nor whether an increased infusion duration was associated with better protection, based on our experimental design. Secondly, the protective effects of EIso (4 ml/kg) and equivalent Iso were not compared on the rat model of CA due to a lack of studies comparing MAC between EIso and Iso in rats. In addition, the blood and end-tidal concentrations of Iso in the Iso and EIso groups were not measured; therefore, unequal doses of Iso may have been administered to rats. Thirdly, transient global ischemia induces neuronal damage, particularly in the CA-1 region of a rat's hippocampus. Therefore, the brain morphology analysis of the present study was only restricted to the CA-1 region (41). Moreover, the cortex, thalamus and striatum were not evaluated in detail due to the difficulty of defining an exact area in these structures. Finally, the EIso postconditioning effect was evaluated using a 6-min asphyxia. Although a previous study has shown that CA induced by 6-min of asphyxia is enough to exhibit ischemic neuronal injury in rats (42), further exploration is required to clarify the protective effect of EIso after a longer period of asphyxia. In addition, an asphyxia-induced CA model was investigated, and therefore nonasphyxial causes, such as ventricular fibrillation, cannot be precluded.

In conclusion, the present study demonstrated that EIso postconditioning improved survival and neurological outcomes on a rat model of CA. Furthermore, the observations of the present study suggested a potentially novel and easily applicable method to treat post-CA syndrome. Therefore, the present study may pave the way for a successful translation of EIso postconditioning into clinical practice.

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