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Original Article

# Protective Effect of Emulsified Isoflurane Preconditioning on Cardiac Toxicity Induced by Bupivacaine in Rats

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## ABSTRACT

**Background:** Lipid emulsion has been identified as a potent rescuing agent for cardiac arrest caused by local anesthetic overdose. In this animal study, we investigated whether prophylactic infusion of emulsified isoflurane, a mixture of lipid emulsion and isoflurane, could increase the tolerability for bupivacaine-induced cardiac toxicity.

**Methods:** Rats were randomly assigned to receive one of the following treatments: saline, 30% Intralipid, 4% Emulsified Isoflurane (4% EISO), 2% EISO, 0.5% propofol, 0.25% propofol, inhaled isoflurane plus 30% Intralipid, or inhaled isoflurane plus saline, for 15 minutes. Then 0.75% bupivacaine was infused at the rate of 8 ml/kg/min (n=10 in each group). The time needed to induce cardiac arrest was recorded and the bupivacaine dose was calculated. Another set of rats were intubated for mechanical ventilation and catheterized for invasive arterial pressure monitoring while receiving one of the following sedative pretreatments for 15 minutes: 4% EISO, 0.5% propofol, inhaled isoflurane plus saline, or inhaled isoflurane plus 30% Intralipid (n=10 in each group). Then bupivacaine was infused at the rate of 8 ml/kg/min for 120 seconds (sublethal dose). The hemodynamic parameters were recorded till circulation fully recovered.

**Results:** Pretreatment with 4% EISO significantly increased the dose of bupivacaine required to induce cardiac arrest (68.69 ± 7.57 mg/kg vs. 26.61 ± 5.13 mg/kg for saline, $P<0.01$). Prophylactic infusion of Intralipid alone also increased the bupivacaine tolerability (51.41 ± 9.68 mg/kg, $P<0.05$ vs. saline), but less efficient than 4% EISO ($P<0.05$ vs. 4% EISO). Pretreatments with 4% EISO provided best preservation of hemodynamic parameters in the face of circulatory fluctuation caused by sublethal dose of bupivacaine.

**Conclusions:** The 4% emulsified isoflurane preconditioning significantly increases the threshold of bupivacaine-induced cardiac arrest in rats and prevents circulatory instability caused by sublethal dose of bupivacaine. Our results implicate the potential application of emulsified isoflurane as an adjuvant agent in local anesthesia.

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As early as in the 1970s, lipid emulsion was investigated as a rescuing agent against toxicity caused by lipophilic drugs. In 1998, Weinberg et al demonstrated that cardiac arrest caused by bupivacaine overdose could be reversed by lipid emulsion in rats (1), which was considered as a milestone in our specialty. Later, the rescuing effect of lipid emulsion was confirmed in various animal models and clinical case reports (2-7). Therefore, lipid emulsion is recommended as the first-line agent to treat cardiac arrest caused by local anesthetics overdose in clinical guideline (8). However, it remains controversial on the timing and dosage of lipid emulsion in this life-threatening situation.

Emulsified isoflurane is prepared by dissolving liquid isoflurane into 30% Intralipid. Phase I clinical trial has demonstrated that emulsified isoflurane is a safe intravenous anesthetic in human, which produced rapid onset and short lasting anesthesia in volunteers in a dose-dependent manner (9). In previous animal study, EISO had a significantly shorter recovery time than propofol after single injection, with a similar onset time, which suggested that EISO was more adjustable. In earlier preclinical studies, it was further showed that emulsified isoflurane was effective in protecting myocardium against ischemia-reperfusion injury (10, 11) .

In this study, we investigated whether that emulsified isoflurane is effective in increase the tolerability against bupivacaine-induced cardiac toxicity caused by bupivacaine, one of the most life-threatening complications in anesthetic practice.

## EXPERIMENTAL MATERIALS AND METHODS

Approved by the Experimental Animal Ethics Committee of Sichuan University, 300-400 g male SD rats were used and maintained for 12 hours as a light-dark cycle. Animals had free access to water and food. All animal studies were performed between 09: 00-16: 00.

Emulsified isoflurane was prepared as previously reported (9). Briefly, liquid isoflurane (Abbott Laboratories, Shanghai) was dissolved into 30% Intralipid (Jiangsu Huarui pharmaceutical companies) at the volume ratio of 1:11.5, which presents an isoflurane concentration of 8% (vol/vol). 100 ml 8% EI contains 8 ml pure liquid isoflurane. Then it was further diluted with 30% Intralipid as needed. Propofol (2,6-diisopropyl phenol, CAS number 2078-54-8, Sigma, China) was dissolved in 30% Intralipid at desired concentrations. 0.75% Bupivacaine hydrochloride was purchased from Shanghai Zhaohui Pharmaceutical.

Mainly used in experimental equipment, including: Smith Medical Micropump; Thai Union BL-420E biological function of experimental recording system for recording the heart rate, blood pressure; Thai Union small animal ventilator (HX-100E); Philips Monitor Radimeter ABL800 blood gas analysis instrument (Radumeter, Denmark), I13L probe (5.8-14.0 MHz).

### Experimental Protocols

*Experiment 1 Effect of Emulsified Isoflurane on the Threshold of Bupivacaine to Induce Cardiac Asystole*

The experimental protocol was present in Figure 1A. The rat was fixed with a fixator to allow easy access to the hind limbs and tail. The hind limbs were prepared and connected to noninvasive pulse oximeter oxygen saturation probe. A 24G catheter was inserted into the tail vein for drug infusion. The rats were randomly assigned to receive one of the following pretreatment: Saline, 30% Intralipid (IL), 4% Emulsified Isoflurane (4% EISO), 2% Emulsified Isoflurane (2% EISO), 0.5% Propofol (0.5% PROP), 0.25% Propofol (0.25% PROP), Inhaled Isoflurane plus 30% Intralipid (ISO + IL), or Inhaled Isoflurane plus Saline (ISO + Saline). For pretreatment, the rate of drug infusion was fixed at 1 ml/kg/min for all the drugs, based on Weinberg's study (1) and our unpublished pilot data. The inhaled isoflurane was given at the concentration of 2%. In our preliminary study, we found that the sedative depth was similar among 4% EI, 0.5% propofol and 2% isoflurane inhaled groups. The pretreatment drugs were infused or/and inhaled for 15 minutes, followed by 0.75% bupivacaine infusion at the rate of 8 ml/kg/min. The time point at which bupivacaine infusion initiated was recorded as T0. After loss of movements following bupivacaine infusion, the rats were removed from the fixator and connected to an

electrocardiogram. Cardiac arrest was defined as asystole ECG waveform for continuous 10 seconds. The duration of bupivacaine infusion to induce cardiac arrest was recorded and the infused bupivacaine dose was calculated. During the whole study, the animals received 100% oxygen via a customized mask.

*Experiment 2 Effect of Emulsified Isoflurane on Hemodynamics after Infusion of Sublethal Dose Bupivacaine*

The experimental protocol was present in Figure 1B. Following the identical procedure in experiment 1, animals received one of the following drug pretreatments: 4% Emulsified Isoflurane (4% EISO), 0.5% Propofol (0.5% PROP), Inhaled Isoflurane plus Saline (ISO + Saline), or Inhaled Isoflurane plus 30% Intralipid (ISO + IL). The pretreatment drugs were given at the same rate or concentration as in experiment 1 for 15 minutes, during which the femoral artery was cannulated and the trachea was intubated. Since Intralipid and saline cannot provide sedation for the invasive procedures, these two pretreatments were not employed in experiment 2.

As soon as bupivacaine infusion began following the 15-minutes treatment, mechanical ventilation was initiated (tidal volume of 3 ml; respiration rate at 60 per minute). Bupivacaine (0.75%) infusion lasted for 120 seconds at the rate of 8 mg/kg/min (16 mg/kg). At the initiation and termination of bupivacaine infusion, as well as 5 minutes and 10 minutes after bupivacaine infusion, cardiac function was evaluated with M-mode echocardiography (GE vivid 4 ultrasound machine). Arterial blood gas was analyzed at the beginning of bupivacaine infusion, which was re-analyzed 10 minutes after bupivacaine infusion.

### Statistical Analysis

PASW statistics 18.0 statistical software (formerly SPSS statistical analysis software) was used for statistical analysis. Hemodynamic parameters included heart rate (HR) and mean arterial pressure (MAP). Cardiac output (CO) was calculated using M-mode echocardiographic measurements. ANOVA was performed for data with normal distribution and Kruskal-Waillis analysis was performed for data with non-normal distribution. Repeated measurements were analyzed using repeated measures analysis of variance. P value equal to or less than 0.05 was regarded as statistical significance.

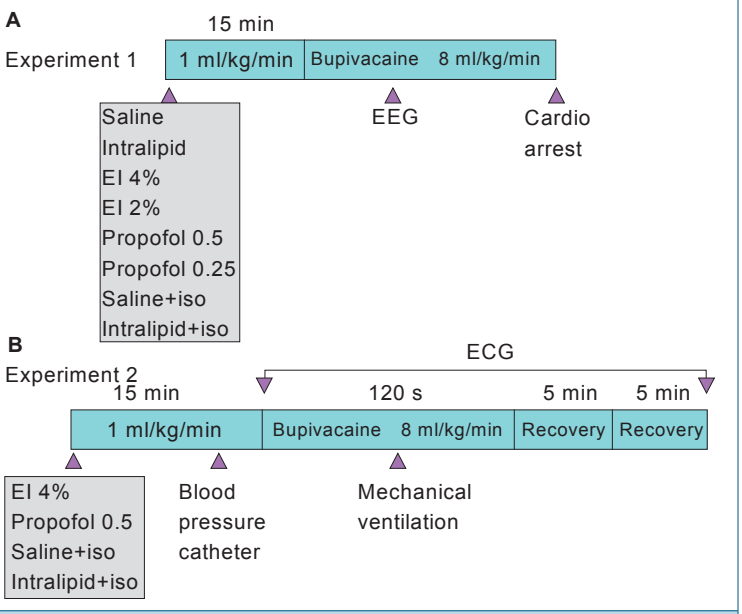


**Figure 1. The Experiment Protocol.**

## RESULTS

Emulsified isoflurane pretreatment increases tolerability against bupivacaine- induced cardiac toxicity.

Compared with saline alone, pretreatments containing the Intralipid component increases the tolerability against bupivacaine-induced cardiac toxicity to some extent, with all their respective P values less than 0.05 compared with saline alone (Table 1). It is interesting to note that compared with animals receiving inhaled isoflurane plus Intralipid infusion, animals receiving 4% emulsified isoflurane require even higher bupivacaine dose to induce cardiac arrest ($68.69 \pm 7.57$ vs. $60.18 \pm 8.30$ mg/kg, $P<0.05$). However, 2% emulsified isoflurane shows no superiority to inhaled isoflurane plus Intralipid infusion to enhance tolerability against bupivacaine- induced cardiac arrest ($60.43 \pm 5.07$ vs. $60.18 \pm 8.30$ mg/kg, $P>0.05$). Inhaled isoflurane added to Intralipid infusion failed to enhance bupivacaine tolerability ($60.18 \pm 8.30$ vs. $51.41 \pm 9.68$ mg/kg, $P>0.05$).

**Table 1. Noninvasive Oxygen Saturation the End of the Pretreatment and Bupivacaine Dose to Cause Cardiac Asystole.**

| | Bupicavaine needed to cause asystole(mg/kg) | $SpO_2$ at the end of the 15-min Pretreament (%) |
|---|---|---|
| Saline | 26.01±5.13 | 98.7±1.0 |
| IL | 51.41±9.68* | 97.7±1.1 |
| ISO+Saline | 27.97±5.88 | 93.3±0.8*† |
| ISO+IL | 60.18±8.30* | 93.7±0.7*† |
| 2% Eiso | 60.43±5.07* | 93.8±0.8*† |
| 4% Eiso | 68.69±7.57*† | 92.7±1.2*† |
| 0.25% Prop | 43.44±6.04*&# | 91.8±2.4*† |
| 0.50% Prop | 41.88±4.75*&# | 89.3±0.8*†&# |

Data were presented as mean±SD; n=10 in each group.
*P<0.01 versus saline alone; †P<0.05 versus IL; &P<0.01 versus 2% EISO; #P< 0.01 versus 4% EISO.

**Table 2. Hemodynamics before and after Infusion of Sublethal Dose of Bupivacaine.**

| | 4% Eiso | 0.5% Prop | ISO+IL | ISO+Saline |
|---|---|---|---|---|
| **Before bupivacaine infusion** | | | | |
| HR (beats/min) | 350±36 | 324±43 | 351±22 | 328±28 |
| MAP (mm Hg) | 124±19 | 119±23 | 135±17 | 126±26 |
| RPP | 52590±7712 | 45987±7913 | 54174±5905 | 46731±5111 |
| **After bupivacaine infusion** | | | | |
| HR (beats/min) | 197±39*& | 143±24*† | 201±23* | 74±31 |
| MAP (mm Hg) | 95±20* | 94±18* | 101±14* | 56±18 |
| RPP | 24564±2311*& | 14887±1543*† | 23232±3648* | 6837±3807 |

Data were presented as mean±SD; n=10 in each group.
*P<0.01 versus ISO+saline; †P<0.01 versus ISO+IL; &P<0.01 versus 0.5% Prop.

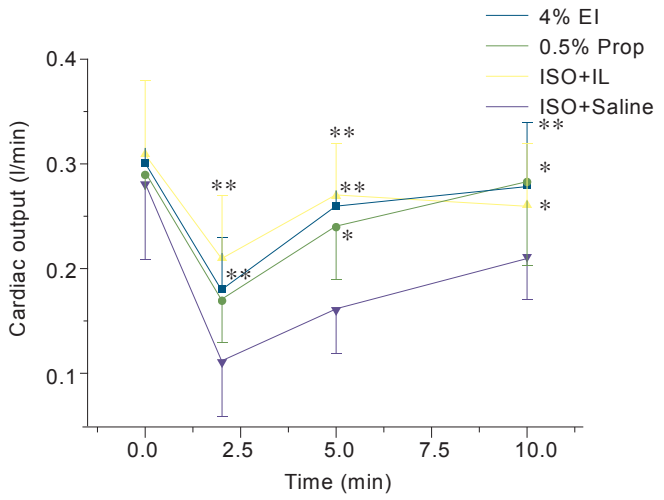


**Figure 2. The Cardiac Output Measured by M-Mode Echocardiograph.**
*P<0.05 vs. ISO+Saline; **P<0.01 vs. ISO+Saline.
EI=Emulsified Isoflurane; Prop=Propofol; ISO=Inhaled Isoflurane; IL=Intralipid.

It is further found that propofol, another commonly used anesthetics in emulsion formulation, is less efficient in enhancing bupivacaine tolerability (41.88 ± 4.75 mg/kg) while compared with either 2% (60.43 ± 5.07 mg/kg, P< 0.05) or 4% (68.69±7.57 mg/kg, P<0.05) emulsified isoflurane.

Administration of propofol, emulsified isoflurane and inhaled isoflurane all produced mild respiratory depression, reflected as significantly decreased oxygen saturation, which remains within the normal range. However, infusion of 0.5% propofol produces more severe respiratory depression as oxygen saturation further drops to 89%.

Since mild respiratory depression was observed animals receiving propofol or isoflurane in experiment 1, mechanical ventilation with 100% oxygen was used in experiment 2 to avoid any possible hemodynamic instability secondary to hypoxia. Since neither 2% EISO nor 0.25% propofol produced sedation deep enough for intubation, both regimens were excluded in experiment 2.

All the animals survived and restored normal rhythm spontaneously. Arterial blood gas analysis performed at the beginning bupivacaine infusion revealed no difference between groups. 10 minutes after initiation of bupivacaine infusion, animals in group ISO + saline had significantly higher level of lactate (2.13±0.55 mmol/L) than those in group 4% EISO (0.93 ± 0.15 mmol/L, P<0.05), and in group ISO + Intralipid (0.87 ± 0.24 mmol/L, P<0.05). There was no difference in any other blood gas parameters, e.g. pH, BE and $HCO_3^-$, between the tested groups.

The heart rate (HR), mean arterial pressure (MAP), and heart rate- systolic blood pressure (RPP) decreased during bupivacaine infusion regardless of pretreatment received (Table 2). Compared animals with ISO+Saline, the animals with other three pretreatments containing the component of emulsion, had no or significantly shorter duration of RPP below the baseline.

With a similar trend to hemodynamic parameter, cardiac output decreased following bupivacaine infusion, which spontaneously returned to baseline following termination of bupivacaine infusion. Pretreatments containing the component of Intralipid resulted in better recovery of cardiac function as showed Figure 2.

## DISCUSSION

In the present study, pretreatment emulsion component increased the threshold for bupivacaine-induced cardiac arrest, and prevented cardiovascular instability caused by sublethal dose of bupivacaine.

Local anesthetic-induced cardiotoxicity could be reversed with lipid emulsion, as first reported by Weinberg GL in 1998, that pre-treatment with fat emulsion can increase the threshold of cardiac arrest caused by bupivacaine. In a series of follow-up studies, the protective effect of emulsion against cardiotoxicity caused by local anesthetics has been confirmed in various animal models. The proposed mechanisms included "lipid sink" theory (12, 13) and improved metabolic status (14).

It is noteworthy to point out that, in the Weinberg's study, all the animals were first exposed to isoflurane for vascular catheterization, before infusion of lipid emulsion or bupivacaine. Therefore, the potential rescuing effect of background isoflurane on bupivacaine-induced cardiac toxicity was neglected, since isoflurane has been confirmed to be a potent cardioprotective agent (15). In our study, 51.41 ± 9.68 mg/kg bupivacaine was required to induce cardiac arrest in conscious animals received 30% Intralipid alone, which was much lower than 82 mg reported from Weinberg with 1.75% isoflurane co-administrated. It cannot be ruled out this difference might result from different experiment sittings because the volume of Intralipid is smaller than the volume used by Weinberg's study. In our current study, animals receiving 4% emulsified isoflurane required 68.69 ± 7.57 mg/kg bupivacaine to induce cardiac arrest, which was significantly higher than that with Intralipid alone. With 30% Intralipid plus inhaled 2% isoflurane, there remained a trend indicating higher dose of bupivacaine was needed to induce cardiac arrest, although this increase did not reach the statistical significance. However, inhaled isoflurane alone failed to increase the tolerability against bupivacaine-induced cardiac toxicity. This could be explained as below: with spontaneous inhalation of isoflurane for 15 minutes, the increase in blood isoflurane concentration was too slow to reach a significant concentration. On contrary, intravenous injection of emulsified isoflurane resulted in an immediately and significantly increase in isoflurane blood level. Taken together, our study indicated that in the presence of lipid emulsion, isoflurane offered extra protection against bupivacaine-induced cardiac toxicity, and the protective effect would be maximized with pre-mixture of lipid emulsion and isoflurane, i.e. with emulsified isoflurane. It is not clear yet why emulsified isoflurane is superior to inhaled isoflurane plus Intralipid. Modification of the formulation might lead to alternation of pharmacodynamical/pharmacokinetical profiles of isoflurane, as pointed out by Lucchinetti (16).

All the commercially available propofol was prepared with 10% Intralipid. To rule out the possible effect of emulsified at different concentrations, propofol used in our current study was freshly prepared with 30% Intralipid. Propofol pretreatment failed to increase the intoxicating dose of bupivacaine, compared with 30% Intralipid alone. And the intoxicating dose was much lower for propofol pretreatment while compared with emulsified isoflurane, which suggest that propofol per se is lack of protection against bupivacaine-induced cardiac toxicity. Therefore, the observed increased bupivacaine intoxicating dose compared with saline is likely to result from Intralipid alone. If commercial propofol used (10% lipid emulsion), the protective effect would be even more negligible.

Our study had important clinical implications. Previous studies focused on using lipid emulsion as a rescuing agent for bupivacaine-induced cardiac toxicity. Our results showed that drugs contained emulsion could be administrated before application of bupivacaine to prevent cardiac arrest. This further justify the use of emulsified isoflurane as an adjuvant agent for local anesthesia, which provide sedation while prevent bupivacaine-induced cardiac toxicity. Furthermore, emulsified isoflurane has shown to produce synergism with lidocaine in regional anesthesia (17) and provide an anti-convulsion effect in lidocaine-induced seizure (18). Thus, emulsified isoflurane might be useful in local anesthesia in clinical setting.

In summary, 4% emulsified isoflurane pre-

treatment significantly increased the threshold of bupivacaine-induced cardiac arrest in rats and prevents circulatory instability caused by sublethal dose of bupivacaine. Other drug in the formulation of emulsion, e.g. propofol, had little protective effect.

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The authors have no conflicts of interest for this work to declare.

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