Association of sustained cardiovascular recovery with epinephrine in the delayed lipid-based resuscitation from cardiac arrest induced by bupivacaine overdose in rats

B. Li††, J. Yan††, Y. Shen†, B. Li†, Z. Hu† and Z. Ma†*

1 Department of Anesthesiology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China
2 Department of Anesthesiology, The Affiliated Nanjing Maternity and Child Health Care Hospital, Nanjing Medical University, Nanjing, China
3 Key Laboratory of Drug Control and Pharmacovigilance, Ministry of Education, Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing, China
* Corresponding author. E-mail: 041104160@fudan.edu.cn

Editor’s key points

- Although lipid is recommended to treat local anaesthetic cardiac toxicity, in some countries, there may be a delay in obtaining the lipid.
- Giving delayed epinephrine plus lipid treatment was investigated in rats given bupivacaine.
- Late administration of epinephrine with lipid improved haemodynamic recovery but worsened hypoxaemia and acidosis.

Background. The role of epinephrine combined with lipid emulsion in rescuing cardiovascular collapse induced by local anaesthetic overdose remains unclear. The objective of this study was to explore the effect of epinephrine on delayed lipid-based treatment for bupivacaine-induced cardiac arrest in rats.

Methods. Thirty-two rats were subjected to bupivacaine to induce asystole. Basic life support was performed for 10 min before the rats received saline, epinephrine alone, or 20% lipid emulsion bolus with or without epinephrine pretreatment. ECG and invasive arterial pressure were monitored continuously. Arterial blood gas was analysed at 25 min; the right lungs and hearts of rats were harvested for measurement of dry-to-wet lung weight ratio and myocardial bupivacaine content, respectively.

Results. In the rats treated with epinephrine plus lipid emulsion, there was a marked improvement in haemodynamic parameters at 25 min compared with rats treated with lipid alone, \( P < 0.05 \). The coronary perfusion pressure immediately after lipid rescue was higher in the epinephrine/lipid-treated rats when compared with rats given lipid only (70 and 24 mm Hg, respectively, \( P < 0.05 \)). The myocardial bupivacaine content was lower (8.34 nM g\(^{-1}\)) in the epinephrine/lipid group relative to other groups (\( P < 0.05 \)). However, the rats treated with lipid alone which survived had higher \( P_O_2 \), less severe acidosis, and better hypoxaemia relative to surviving rats given epinephrine plus lipid.

Conclusions. Late intervention with epinephrine plus lipid emulsion contributed to sustained improvement in haemodynamic profile, but failed to alleviate deterioration of hypoxaemia and acidemia in rats.

Keywords: anaesthetics, local; cardiopulmonary resuscitation; epinephrine; fat emulsions, i.v.; heart arrest
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Cardiovascular collapse is the life-endangering complication of local anaesthetic absorption or intravascular injection during regional anaesthesia, generally considered to be resistant to conventional modes of resuscitation.\(^1\)\(^2\) There is growing evidence from animal studies and clinical case reports supporting that the use of lipid emulsion as effective and superior to vasoactive drugs in reversal of bupivacaine-induced cardiac arrest.\(^3\)\(^-\)\(^6\) Moreover, results from Weinberg’s group indicate that high doses of epinephrine (>10 \( \mu \)g kg\(^{-1}\)) actually impair the effect of lipid-based treatment for bupivacaine-induced myocardial toxicity.\(^7\) The recent study by Harvey and colleagues,\(^8\) however, demonstrated that the increase in coronary perfusion pressure (CPP) due to epinephrine pretreatment was a prerequisite for the efficacy of i.v. lipid emulsion (ILE) in rescuing asystole induced by bupivacaine in rabbits. In addition, ILE infusion is usually commenced immediately after systole in most animal studies. However, lipid emulsion is not routinely stocked and may not be available in operating theatres in China, thus potentially delaying its administration. Notwithstanding, there are several clinical reports stating ILE still can be used successfully to reverse the myocardial toxicity even after prolonged conventional cardiopulmonary resuscitation (CPR) has failed, the efficacy of late intervention with
ILE for bupivacaine-induced cardiac arrest remains unclear, and the role of epinephrine in the delayed lipid-based treatment has not been investigated to date.\textsuperscript{9, 10} We postulated that epinephrine could promote an increase in CPP and benefit lipid emulsion transportation to myocardial tissue during prolonged CPR rescue from local anaesthetic overdose.

In the present study, rats were subjected to bupivacaine-induced cardiac arrest at a dose of 30 mg kg\textsuperscript{-1}, followed by basic life support for 10 min before ILE with or without epinephrine pretreatment (10 μg kg\textsuperscript{-1}). The haemodynamic and metabolic parameters and the bupivacaine concentration in cardiac tissue were evaluated and the underlying mechanism of the reversal of myocardium intoxication by ILE was also investigated.

### Methods

#### Experimental animals

Healthy male Sprague–Dawley rats (7 week old) were purchased from Xipuer-Bikai laboratory animal Co., Ltd (Shanghai, China) and housed in dry-raising cages (six rats per cage) at the animal centre of the Affiliated Hospital of Nanjing University Medical School, in a standard 12 h reverse day/night cycle at an ambient temperature of 26°C. The rats had an ad libitum diet of stock laboratory diet and tap water. They had a stabilization period of 1 week before experimentation.

#### Animal model and resuscitation protocol

The following protocol was approved by the Institutional Animal Care and Utilization Committee of Nanjing University Medical School. The 32 rats were randomized using a random number protocol with Excel 2003 and divided into four groups of eight rats in each group (Microsoft, USA). Rats weighing between 180 and 294 g were anaesthetized with ketamine hydrochloride (150 mg kg\textsuperscript{-1} 1 i.p.). Subsequently, the rats were orally intubated under direct view and mechanical ventilation was commenced with 100% oxygen using rodent volume-controlled ventilation (Teli Company, Jiangxi, China): tidal volume 8 ml kg\textsuperscript{-1}, respiratory rate of 60 bpm, and an inspiration:expiration ratio of 1:1. The right external jugular vein and the right internal carotid artery were cannulated and connected to a pressure transducer of Eagle400 monitor (GE Marquette Medical, USA). Body temperature was maintained at 38–39°C with a heating lamp held at a safe distance. ECG invasive arterial pressure and external jugular venous pressure were recorded continuously throughout the experiment using an Eagle400 Monitor.

If the systolic arterial pressure was >150 mm Hg for over 2 min during the stabilization phase, another dose of ketamine (50 mg kg\textsuperscript{-1} 1 i.p.) was administered to ensure adequate anaesthesia. After 5 min stabilization, 30 mg kg\textsuperscript{-1} bupivacaine was delivered i.v. over 30 s. All rats developed asystole by the end of the infusion (zero time). Manual chest compressions (compressing the middle of the rat’s sternum with the thumb and index finger at the rate of 300 min\textsuperscript{-1} to produce a systolic arterial pressure of ~30 mm Hg) were started immediately and delivered until return of spontaneous circulation (taken as a native rate-pressure product (RPP) = systolic pressure×heart rate, >20% of the baseline value for 2 min). The tidal volume was adjusted to 12 ml kg\textsuperscript{-1} and 5% NaHCO\textsubscript{3} (0.2 ml kg\textsuperscript{-1}) was given intermittently every 5 min to alleviate systemic acidosis from the 5 min time point to the end of the experiment. Chest compressions were interrupted for 5 s each minute to assess native RPP.

Animals (n=8 for each group) were randomly assigned in advance to receive one of four treatments according to the following regimens: epinephrine (E) group: rats received an epinephrine bolus (10 μg kg\textsuperscript{-1}) at 1, 3, 5, 10 min followed by intermittent bolus every other 3 min until RPP>20% of the baseline value. Saline (S) group: the rats were given saline (1 ml kg\textsuperscript{-1}) at 1, 3, 5 min, then a saline bolus (5 ml kg\textsuperscript{-1}) at 10 min followed by an infusion at the rate of 0.5 ml kg\textsuperscript{-1} min\textsuperscript{-1}. Lipid (L) group: rats received the same treatment as the saline control rats except that saline was replaced by lipid emulsion (20% Intralipid, Huarui Pharmaceuticals Co., Ltd, China, 5 ml kg\textsuperscript{-1}) at 10 min followed by continuous lipid infusion at the same rate (0.5 ml kg\textsuperscript{-1} min\textsuperscript{-1}). Epinephrine plus lipid (EL) group: rats received the same treatment as the lipid-treated group rats except that 10 μg kg\textsuperscript{-1} epinephrine was delivered immediately before the lipid emulsion treatment at 10 min. After the initial dose of medication 10 min after cardiac arrest, repeated medications were used at 12 and 15 min until the RPP reached 20% of the baseline value. Arterial blood samples (0.3 ml) were drawn at baseline and at the end of experiment for blood gas analysis and serum lactate measurement. Thereafter, all animals were killed by exsanguination and the lungs were dissected for determination of wet-to-dry lung weight ratio. The hearts were harvested and stored at −80°C before homogenization for measurement of cardiac tissue bupivacaine content using high-performance liquid chromatography (HPLC). The experiments were not blinded, but data were analysed by personnel blinded to the treatment group.

#### Cardiac tissue bupivacaine content

Frozen hearts (500 mg) were homogenized with 1 ml saline and centrifuged at 4000 rpm for 15 min, then 0.2 ml supernatant was collected and mixed with 0.6 ml methanol:acetonitrile (1:1) solution and vortexed for 3 min. After the mixed solution was centrifuged at 14,000 rpm for 5 min, the supernatant was collected and injected into the HPLC system. HPLC was performed on a SHIMADZU 10ATyp system (Kyoto, Japan) with a C18 column (4.6×150 mm, 5 μm, Lichrospher C18, Hanbon, Huaian, China) and 5 mM ammonium:acetonitrile (63:37) as mobile phase. The flow rate was 1.0 ml min\textsuperscript{-1} and subsequent detection was by UV absorbance at 220 nm. The limit of quantification for bupivacaine was 0.62 nM g\textsuperscript{-1}. The retention time for bupivacaine was 8.0 min. Recovery of bupivacaine from cardiac tissue samples ranged from 76.7 to 92.2% with an inter-assay precision of <9.7% coefficient of variation.
Statistical analysis

Power analysis was based on results of preliminary experiments comparing RPP at 25 min among various treatment groups; specifically, power was set at 0.8, significance criteria was set at 0.05, effect size was estimated as 2, and sigma at 0.9. This yielded a sample size of n=8 for each group. Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). All haemodynamic parameters were compared across time by two-way analysis of variance with repeated measures and Bonferroni post-tests when significance was achieved (α set at 0.05) for differences over time within and between groups. Baseline parameters, major metabolic parameters, CPP, and wet-to-dry lung weight ratio were analysed by one-way analysis of variance and Bonferroni post-tests when indicated by significance of difference. Data were normally distributed and are therefore presented as mean (SD). The myocardial lysis of variance and Bonferroni post-tests when indicated by significance was achieved (α set at 0.05) for differences between the groups.

Results

Baseline values: no differences were observed between groups in baseline haemodynamic and metabolic metrics (Table 1).

Cardiac function measurement

All rats rapidly developed cardiac arrest at the end of 0.75% bupivacaine (30 mg kg⁻¹) i.v. infusion (zero time). RPP was the key metric of cardiac function in this model. Recovery in terms of mean RPP (so) against time is shown in Figure 1. The RPP reached maximal value and approximated 6000 mm Hg min⁻¹ at 10 min, then reduced markedly during the subsequent 15 min period in the epinephrine group. The RPP in the lipid group steadily increased after lipid emulsion treatment, but was below 6000 mm Hg min⁻¹ at 25 min. In contrast, RPP markedly increased to more than 7000 mm Hg min⁻¹ 5 min after combined epinephrine and lipid treatment and reached 11 149 mm Hg min⁻¹ at 25 min (P<0.01). None of the rats in the saline group survived (defined in advance of study as RPP > 20% baseline for at least 5 min until 25 min time point without extra medication support). In contrast, five of eight rats in the epinephrine/lipid group, two rats from the epinephrine (n=2) and three from the lipid group, were successfully rescued by 25 min. As shown in Figure 1, mean arterial pressure (MAP) and heart rate in epinephrine/lipid-treated rats were higher compared with rats in the other groups at 12, 15, 20, and 25 min (P<0.05).

Coronary perfusion pressure

CPP (defined as simultaneously recorded diastolic arterial pressure minus external jugular venous pressure) after saline or epinephrine rescue treatment at 11 min is presented graphically in Figure 2. The mean (so) CPP of rats treated with epinephrine [61 (9.5) mm Hg] or epinephrine/lipid group [70 (9.6) mm Hg] were markedly higher compared with that of saline [28 (2.5) mm Hg] or lipid-treated rats [24 (8.3) mm Hg] (P<0.05).

Cardiac tissue bupivacaine content

The bupivacaine concentration in cardiac tissue at 25 min in the four different groups is depicted in Figure 3. The results demonstrated that the bupivacaine content in the epinephrine/lipid-treated rats was much lower than in the other groups (P<0.05).

Metabolic parameters

Bupivacaine-induced cardiac arrest resulted in a dramatic reduction in pH and severe acidosis in epinephrine, lipid, or epinephrine plus lipid-treated rats by 25 min (no blood samples were able to be drawn for further analysis in saline-treated rats as no animals in this group attained ROSC at 25 min). As shown in Table 2, the rats in the epinephrine group exhibited more severe acidosis (BE < −15 mmol litre⁻¹), hypoxaemia, and a significant increase in Pco₂ compared with rats treated with lipid only (P<0.01). Consistent with previous studies, the rats surviving in the lipid group (n=3) exhibited better oxygenation and less severe acidemia compared with epinephrine plus lipid (P<0.05). Strikingly, the Pco₂ in the lipid group rats approximately double that of rats given

| Table 1 | Baseline values of key parameters in all four groups. All values are mean (so). Baseline values for major parameters showed no difference among the four groups. MAP, mean arterial pressure; RPP, heart rate pressure product; Lac, lactate concentration in blood; BE, base excess |
|---|---|---|---|---|
| | Saline (n=8) | Epinephrine (n=8) | Lipid only (n=8) | Epinephrine + lipid (n=8) |
| Weight (g) | 242 (30) | 226 (25) | 234 (37) | 232 (16) |
| MAP (mm Hg) | 124 (22) | 118 (21) | 117 (22) | 115 (18) |
| RPP (mm Hg beats min⁻¹) | 37 350 (6502) | 35 325 (6169) | 35 025 (6596) | 34 613 (5539) |
| PH | 7.45 (0.03) | 7.47 (0.02) | 7.43 (0.02) | 7.45 (0.01) |
| Po₂ (kPa) | 53.6 (3.5) | 57.6 (5.7) | 55.3 (3.9) | 56.3 (4.5) |
| Pco₂ (kPa) | 4.3 (0.6) | 4.6 (0.7) | 4.7 (0.7) | 3.9 (0.5) |
| Lac (mmol litre⁻¹) | 1.23 (0.34) | 0.93 (0.40) | 1.1 (0.07) | 1.21 (0.12) |
| BE (mmol litre⁻¹) | −5.2 (0.36) | −4.3 (0.42) | −4.0 (0.44) | −6.0 (0.72) |
Fig 1  Haemodynamic changes during resuscitation. Mean (SD) arterial pressure (A), heart rate (B), and native RPP (C) are plotted against time. Two-way analysis of variance with repeated measures and Bonferroni post-tests were used to compare mean differences over time within and between groups. Significance of differences is shown for epinephrine plus lipid vs saline (*), epinephrine vs saline (+), lipid vs saline (†), epinephrine plus lipid vs lipid only (#). **P, 0.01, #P, 0.05, ##P, 0.05, †P, 0.05, ++P, 0.01; n=8 for each group.

Fig 2  Mean (SD) CPP at 11 min. One-way analysis of variance followed by Bonferroni post-tests was used to compare mean values among various groups. Significance of differences is also shown for epinephrine plus lipid vs saline (P=0.002) or lipid only (P=0.005) and epinephrine only vs saline (P=0.017) or lipid only (P=0.036), n=8 for each group.

Fig 3  Median (IQR) myocardial bupivacaine content at 25 min. The Kruskal–Wallis test followed by Dunn’s post-test was used to compare the median values. Significance of differences is also shown for epinephrine plus lipid vs saline, lipid only, or epinephrine only (*). *P<0.05, n=8 for each group.
epinephrine plus lipid (P<0.05). No significant difference in blood lactate was observed at 25 min.

**Pulmonary effect**

No significant difference in lung dry-to-wet ratio was observed among different treatment groups (n=8) by 25 min (Fig. 4A). However, wet-to-dry lung weight ratio in the surviving rats in the epinephrine and epinephrine/lipid groups was higher than in animals treated with lipid only (Fig. 4B, P<0.05).

**Discussion**

The results in this study indicate that epinephrine combined with lipid emulsion is effective and superior to lipid emulsion alone in restoring cardiac function in rats, despite the 10 min delay after asystole induced by bupivacaine overdose. Epinephrine pretreatment plus lipid emulsion increased CPP and accelerated removal of bupivacaine from cardiac tissue into ‘lipid sink’ in rats, which plays a key role in sustained haemodynamic improvement. However, the blood gas analysis at 25 min shows that the rats surviving in lipid group have higher PO2, lower PCO2, and less acidosis compared with rats given epinephrine plus lipid.

I.V. lipid emulsion for rescue from local anaesthetic systemic toxicity has been extensively investigated recently in several animal models.11–13 The rat model of bupivacaine overdose (20 mg kg–1) is most commonly used. In our pilot study, all rats, which were given bupivacaine (20 mg kg–1) and treated with intermittent epinephrine (10 μg kg–1) boluses, had restoration of spontaneous circulation at a mean time of 9.3 min and achieved sustained improvement in haemodynamic parameters more than 20 min, without obvious pulmonary oedema (data not shown). A previous study by Ohmura and colleagues14 showed that epinephrine resulted in a higher successful resuscitation rate (92%) in rats where asystole was induced by infusion of bupivacaine at 2 mg kg–1 min–1 for 20 min [cumulative dose of bupivacaine required to produce asystole is 39.6 (9) mg kg–1]. Weinberg first used the rat model of bupivacaine overdose via bolus administration of bupivacaine at the dose of 20 mg kg–1. The results in that study also showed that four of five rats

### Table 2

<table>
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<th>Saline (n=0)</th>
<th>Epinephrine (n=2)</th>
<th>Lipid only (n=3)</th>
<th>Epinephrine + lipid (n=5)</th>
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<td>pH</td>
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<td>P02 (kPa)</td>
<td>6.4 (2.1)</td>
<td>20.4 (4.1)*#</td>
<td>10.4 (3.5)*</td>
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<tr>
<td>PCO2 (kPa)</td>
<td>14.0 (2.4)</td>
<td>8.0 (1.5)*+</td>
<td>11.3 (2.0)</td>
<td></td>
</tr>
<tr>
<td>Lac (mmol litre–1)</td>
<td>11.0 (3.4)</td>
<td>12.3 (4.4)</td>
<td>10.5 (4.1)</td>
<td></td>
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<tr>
<td>BE (mmol litre–1)</td>
<td>&lt;−15</td>
<td>−6.8 (2.4)*#</td>
<td>−14.9 (4.0)</td>
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</table>

**Table 2** The changes of major metabolic parameters in four different groups. All values are mean (so). Lac, lactate; BE, base excess. Significance of differences is shown for lipid only vs epinephrine + lipid (#), lipid only vs epinephrine only (+), epinephrine alone vs epinephrine and lipid (*). *P<0.05, #P<0.05, +P<0.05, ++P<0.01

![Fig 4](image-url) Mean (so) wet-to-dry lung weight ratio at 25 min. (A) Lung specimens from all rats in each group (n=8); (a) lung specimens from surviving rats given epinephrine only (n=2), lipid only (n=3), and epinephrine plus lipid (n=5). One-way analysis of variance followed by Bonferroni post-tests was used to compare mean values among groups. Significance of differences is shown for lipid only vs epinephrine only (P=0.042) or lipid vs epinephrine plus lipid (P=0.023).
treated with epinephrine and two of five rats receiving saline achieved restoration of spontaneous circulation, notwithstanding most animals treated with epinephrine developed severe lung oedema and acidosis within 10 min. A recent study by Kiuchi and colleagues indicated that young Wistar rats showed a remarkable decrease in susceptibility to cardiodepression induced by bupivacaine, and the lethal dose was nearly two times higher in neonatal rats than 16-week-old rats. We presume that the relative susceptibility to epinephrine in bupivacaine-induced cardiac arrest in rodents together with the juvenile rats we used might account for the high survival rate and sustained cardiac recovery in our preliminary study. Therefore, a larger dose of 30 mg kg\(^{-1}\) was used in the present study. During the 25 min observation period after asystole, none of the rats achieved spontaneous circulation with saline treatment and basic life support. Moreover, only 25% of the rats treated with intermittent administration of epinephrine in more clinically relevant manner recovered within 25 min, which indicates that the myocardial intoxication induced by 30 mg kg\(^{-1}\) bupivacaine in rats is relatively resistant to epinephrine treatment, and it is reasonable to proceed to investigate the combined effect of epinephrine and lipid treatment in this model.

Unlike previous studies, we initiated the lipid treatment with or without epinephrine 10 min after cardiac arrest in the present study. To our knowledge, the effect of epinephrine on delayed lipid-based resuscitation from bupivacaine overdose has not previously been investigated. Our results showed that the RPP, heart rate, and MAP were higher in rats given epinephrine plus lipid compared with saline, epinephrine, or lipid alone. In disagreement with Weinberg and colleagues’ study, the rats treated with lipid in the present study did not exhibit the significant and steady cardiovascular recovery compared with epinephrine/lipid treatment, regardless of whether this was immediate or 15 min after administration of lipid. The disparities between the two studies can be attributed to late intervention with lipid in this study. There is considerable evidence supporting the concept of a ‘lipid sink’ mechanism underlying reversal of cardiac arrest by lipid after local anaesthetic overdose. The sink hypothesis proposes sequestration of lipophilic drugs into an expanded plasma lipid phase, with resulting reduction in toxicity. In the absence of sufficient circulation, however, not only is perfusion inadequate to support return of spontaneous circulation, but flow remains insufficient to effect toxin washout. In the present study, the CPP in epinephrine/lipid-treated rats was higher than rats given lipid only within 2 min after medication. Epinephrine/lipid-treated rats consequently had a much lower bupivacaine concentration in cardiac tissue (8.34 nM g\(^{-1}\)); more than 50% reduction when compared with rats given lipid only. Previous studies had shown that lipid emulsion facilitated removal of bupivacaine in cardiac tissue and promoted the restoration of cardiac contractility and conduction in a concentration-dependent manner in the isolated rat heart. The effect of increasing the coronary blood flow by epinephrine can be easily simulated by altering the perfusion flow in the aforementioned in vitro study. Therefore, measures to optimize myocardial perfusion in lipid-based resuscitation from local anaesthetic-induced cardiotoxicity must be considered essential. Our data support the use of epinephrine to guarantee adequate CPP and coronary blood flow in those rats.

To date, however, the optimal dose of epinephrine in lipid-based resuscitation from bupivacaine-induced asystole is still unclear and previous several animal studies had conflicting results. The recent study by Weinberg indicated that a dose of epinephrine >10 \(\mu g\) kg\(^{-1}\) given with lipid was associated with transient cardiovascular recovery (<5 min) and prompt deterioration in haemodynamic profile and tissue metabolism in bupivacaine-overdosed rats, which can be attributed to intense generalized vasoconstriction and oedema of lung parenchyma. Moreover, accumulating evidence indicates that a high dose of epinephrine does not actually increase the survival rate of patients with cardiac arrest and is associated with myocardial stunning after resuscitation. In contrast, recent studies by Mauch and colleagues reported that a conventional dose of epinephrine (10–20 \(\mu g\) kg\(^{-1}\)) is superior to lipid in rescuing piglets from local anaesthetic toxicity. In the present study, we chose the dose of epinephrine commonly used in clinical resuscitation protocols (cumulative maximal dose of epinephrine in the epinephrine/lipid group was not >30 \(\mu g\) kg\(^{-1}\)). In this treatment regimen, epinephrine did not cause severe pulmonary oedema in rats. Moreover, when combined with lipid emulsion, epinephrine facilitated cardiac recovery from asystole induced by bupivacaine overdose in rats. Our present findings were supported by very recent in vitro and in vivo studies. However, the adverse effect of epinephrine at this dose cannot be ignored. The remarkable hypoxaemia and severe acidaemia were obviously associated with epinephrine bolus in epinephrine/lipid-treated animals. The results of wet-to-dry lung weight ratio demonstrated that surviving rats given lipid only developed less severe pulmonary interstitial oedema compared with epinephrine only or epinephrine/lipid-treated animals. The higher dose of epinephrine probably led to myocardial dysfunction after resuscitation and lung interstitial oedema formation, which accounted for hypoxaemia and severe acidaemia.

There are limitations of our study. First, after 10 min external chest compressions, atelectasis in the middle and inferior lobes of the lung, and interstitial lung oedema developed, which resulted in severe reduction in lung compliance. This was testified by observation of less movement of the rat’s thorax and subsequent direct inspection of lungs via thoracotomy. Owing to the inability to adjust PEEP with our rodent ventilator, we therefore adjusted tidal volume to improve ventilation efficiency. This may have accelerated development of the systemic inflammatory response syndrome and circulatory deterioration. We therefore used a 25 min observation period in the present study. Secondly, we did not investigate the concentration–response relationship of epinephrine in the lipid-based resuscitation of asystole in this study, and so we are unable to exclude the possibility that a lower dose of epinephrine (<10 \(\mu g\) kg\(^{-1}\)) may result
in better improvement in metabolic parameters and concomitantly no marked reduction in cardiac function recovery in this model.

In conclusion, the results in the present study imply that late intervention with epinephrine combined with lipid emulsion is effective and superior to lipid treatment alone in reversal of cardiovascular collapse induced by bupivacaine overdose. However, although rats treated with epinephrine plus lipid showed sustained improvement in haemodynamic parameters, the alleviation of deterioration in blood gas was not so evident as lipid group rats relative to epinephrine-treated rats. As far as the significant cardiovascular recovery in animals given epinephrine plus lipid is concerned, the use of epinephrine in lipid-based therapy is suggested, especially when treatment of myocardial intoxication induced by bupivacaine is delayed.

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**Declaration of interest**

None declared.

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