Resuscitation with lipid, epinephrine, or both in levobupivacaine-induced cardiac toxicity in newborn piglets†

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Editor’s key points

- Lipid emulsion with or without epinephrine is useful for treating local anaesthetic (LA) toxicity.
- Studies in newborn animals are needed since neonates are more susceptible to LA toxicity.
- Levobupivacaine was used in newborn piglets to induce cardiovascular collapse.
- Lipid emulsion with or without epinephrine, or epinephrine alone improved survival.
- Lipid emulsion alone was associated with few cardiovascular events.
- Lipid emulsion may be safer in neonates.

Background. The optimal dosing regimens of lipid emulsion, epinephrine, or both are not yet determined in neonates in cases of local anaesthetic systemic toxicity (LAST).

Methods. Newborn piglets received levobupivacaine until cardiovascular collapse occurred. Standard cardiopulmonary resuscitation was started and electrocardiogram (ECG) was monitored for ventricular tachycardia, fibrillation, or QRS prolongation. Piglets were then randomly allocated to four groups: control (saline), Intralipid alone, epinephrine alone, or a combination of Intralipid plus epinephrine. Resuscitation continued for 30 min or until there was a return of spontaneous circulation (ROSC) accompanied by a mean arterial pressure at or superior to the baseline pressure and normal sinus rhythm for a period of 30 min.

Results. ROSC was achieved in only one of the control piglets compared with most of the treated piglets. Mortality was not significantly different between the three treatment groups, but was significantly lower in all the treatment groups compared with control. The number of ECG abnormalities was zero in the Intralipid only group, but 14 and 17, respectively, in the epinephrine and epinephrine plus lipid groups (P < 0.05).

Conclusions. Lipid emulsion with or without epinephrine, or epinephrine alone were equally effective in achieving a return to spontaneous circulation in this model of LAST. Epinephrine alone or in combination with lipid was associated with an increased number of ECG abnormalities compared with lipid emulsion alone.

Keywords: local anaesthesia; toxicity, local anaesthetics

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Local anaesthetic systemic toxicity (LAST) effects have been described in both adults and infants.1–4 Animal and human studies have demonstrated that cardiopulmonary resuscitation after bupivacaine-induced toxicity is difficult;5 6 these findings ultimately led to the clinical development of less toxic local anaesthetics (LAs) such as levobupivacaine and ropivacaine. Levobupivacaine is less toxic than bupivacaine, as confirmed by electrophysiology studies, thus making it one of the LAs of choice in paediatric patients.7

Recently, case reports and experimental studies have supported the use of lipid emulsion, epinephrine, or both as an adjunct to advanced life support during the resuscitation of LA-induced cardiovascular collapse.8–12 Most of these studies did not include newborn animals and used bupivacaine to induce LAST.13 14 It has been shown that children are more sensitive than adults to direct depression of heart contractility by bupivacaine.15 16 Furthermore, studies examining the age-related effects of epinephrine demonstrated that responses in myocardial contractility are reduced in newborn animals.17 Finally, lower concentrations of plasma proteins for drug binding in neonates compared with adults, particularly α-1-acid glycoprotein, increase the amount of free LA available for potential toxicity.

We, therefore, assessed the effects of a lipid emulsion, epinephrine, and a lipid and epinephrine combination on achieving return of spontaneous circulation (ROSC) in anaesthetized newborn piglets that had suffered cardiovascular collapse initiated by toxic doses of levobupivacaine.
Methods

Animal preparation

Ethical approval was granted by the Institutional Animal Care Ethics Committee of the Veterinary School of the University of Lyon (France). The experiments were conducted in an authorized animal laboratory and under the supervision of authorized researchers.

Newborn piglets of either sex were used. The piglets were premedicated with an i.m. injection of xylazine (1.5 mg kg\(^{-1}\), Ronpum\(^{\text{®}},\) Bayer Santé, France) and ketamine (10 mg kg\(^{-1}\), Ketamine, Laboratoire Virbac, France). All animals were placed on warming mattresses to keep their rectal temperature above 37°C. Anaesthesia was induced using 5% inhaled isoflurane via a face mask. Tracheal intubation was performed, the piglets’ lungs were ventilated, and anaesthesia was maintained with 1–2% isoflurane in 100% oxygen. The inspired concentration of isoflurane was continuously monitored and ventilation was adjusted to maintain an end-tidal expired pressure of carbon dioxide between 4.0 and 6.5 kPa.

I.V. morphine (0.1 mg kg\(^{-1}\), Laboratoire Aguettant, Lyon, France) was administered via an ear vein before gaining surgical access of the femoral artery; this allowed continuous monitoring of arterial pressure. The arterial pressure and electrocardiogram (ECG) were continuously monitored throughout (Hewlett-Packard, Andover, MA, USA) and stored digitally for computer processing (MP100 analogue-to-digital apparatus, Acknowledge v.3.03 software; Biopac Systems, Inc., Santa Barbara, CA, USA). The number of ECG abnormalities (ventricular arrhythmias or conduction disorders lasting >30 s) was assessed using the ECG trace. Ventricular tachycardia (VT) was defined as a run of at least four uniform, repetitive ventricular extrasystoles. Ventricular fibrillation (VF) was defined as a persisting non-uniform ventricular arrhythmia. Conduction disorders, such as types 1, 2 and 3 or complete heart block, and QRS prolongation (>120 ms) lasting >30 s were recorded.

Additional data collected included: \(\text{SpO}_2\) and \(\text{EtCO}_2\) (DS 7100, Fukuda Denshi, Japan). A continuous infusion of lactated Ringer’s solution was infused at 10 ml kg\(^{-1}\) h\(^{-1}\) throughout the experiment.

After induction of anaesthesia, the animals were allowed to rest for at least 15 min, before 10 min of baseline data were collected. Once the baseline measures were completed, the isoflurane was discontinued, continuing with ventilation in 100% oxygen alone, and levobupivacaine 2.5 mg ml\(^{-1}\) (or 8.3 mg min\(^{-1}\)) was infused via a peripheral i.v. catheter at a rate of 200 ml h\(^{-1}\) until cardiovascular collapse occurred. Collapse was defined as a 50% decrease in the baseline mean arterial pressure (MAP) for a minimum of 15 consecutive seconds. Cardiovascular collapse was treated using standard cardiopulmonary resuscitation combined with saline or drugs, according to their randomly allocated groups as follows.

**Standard resuscitative procedure**

All the piglets received standard cardiopulmonary resuscitation, consisting of external manual chest compressions at a rate of 100 min\(^{-1}\) and manual ventilation with 100% oxygen. The procedure was only interrupted for a period of <15 s every 2 min, to assess MAP. During the resuscitation attempt, continuous invasive arterial pressure, heart rate (HR), and rhythm disorders were recorded. The primary outcome was ROSC, defined as an unassisted pulse with a return of MAP at or above baseline, for a minimum of 10 min. The resuscitation was stopped if no ROSC was observed after 30 min of CPR.

The animals were killed at the end of the experiment with an overdose of pentobarbital.

Four study groups

The piglets were randomly assigned to one of four groups, a control group and three treatment groups:

- **Control group**: only the standard cardiopulmonary resuscitation was performed and saline was injected.
- **Lipid emulsion group** (LIP): standard cardiopulmonary resuscitation was performed followed by i.v. injection of lipid emulsion (Intralipid 20%, Fresenius Kabi, France) with an initial bolus of 4 ml kg\(^{-1}\) given over 1 min followed by a continuous infusion at a rate of 0.25 ml kg\(^{-1}\) min\(^{-1}\).
- **Epinephrine group** (EPI): standard cardiopulmonary resuscitation was performed followed by an i.v. bolus of epinephrine 10 µg kg\(^{-1}\) repeated every 3 min.
- **EPI and LIP**: standard cardiopulmonary resuscitation, followed by lipid emulsion and epinephrine, as per the EPI and LIP groups.

Statistical analysis

Sample size calculation was based on the results of previous experiments evaluating the effect of epinephrine, lipid, or both on ROSC after LAST. In a Cochran–Armitage test for trend in proportions,\(^{18}\) sample sizes of 7, 7, 7, and 7 were obtained from four groups with X-values equal to 0.0, 0.0, 1.0, and 1.0 (1 lipids and 0 no lipid) and proportions equal to 0.90, 0.70, 0.30, and 0.20 (survival rate obtained from previous studies), respectively. The total minimum sample of 28 subjects achieved 85% power to detect a linear trend using a two-sided Z-test with continuity correction and a significance level of 0.05. To obtain at least 7 piglets in each group, we consecutively randomized all newborns of 3 sows (33 piglets) to one of the four groups using a computer-generated random numerical list. For survival rate analysis, the Marascuolo procedure was used to make comparisons between all pairs of groups.

Baseline parameters were analysed using one-way analysis of variance (ANOVA) after assessment of the normality of the distribution of the data using a Shapiro–Wilk’s W-test. Post hoc tests were used only if ANOVA analysis was significant.

The prevalence of ECG abnormalities was compared with the Fisher exact test. Data are presented as mean (SD) and statistical significance was set at \(P<0.05\).

Results

Data from 33 animals were analysed. Groups were similar in terms of body weight, age, baseline MAP, and HR (Table 1).

The mean cumulative dose of levobupivacaine required to produce cardiovascular collapse was 14.1 (5.5) mg kg\(^{-1}\). Total
doses of levobupivacaine producing collapse were 25.3 (5.9) mg, 33.5 (5.4) mg, 31.3 (13.4) mg, and 30.1 (10.8) mg in the control, EPI, LIP, and EPI–LIP groups, respectively, which was not statistically different. Mean doses of lipid emulsion were 5.5 (1.8) ml kg⁻¹ in the EPI group and 4.6 (0.7) ml kg⁻¹ in the EPI–LIP group (P<0.05). Duration of cardiopulmonary resuscitation received by all animals that achieved a ROSC is given in Table 2, and was not statistically different between groups.

All animals developed dysrhythmias before cardiovascular collapse. Table 3 highlights the number of ECG abnormalities recorded after ROSC. The LIP group had no statistically significant ECG abnormalities after ROSC. This is in contrast to the EPI and EPI–LIP groups, in which ECG abnormalities were detected in, 5 of 6 and 8 of 10 surviving piglets, respectively (P<0.01).

Survival for the different groups is summarized in Table 4. There were no significant differences in survival between the LIP, EPI, and EPI–LIP groups. Survival was significantly better in all the treatment groups compared with the control group.

The mean dosage of EPI required was 45.7 µg kg⁻¹ in the EPI group compared with 12.7 µg kg⁻¹ in the EPI–LIP group (P<0.001). Two piglets in the EPI group had further episodes of cardiovascular collapse after the initial ROSC. These further events were treated as per their original randomization group. No further episodes of cardiovascular collapse were observed in the EPI + LIP and LIP groups.

**Discussion**

Our study shows that the three treatment regimens are equally effective when used during the cardiopulmonary resuscitation of newborn piglets with cardiovascular collapse secondary to levobupivacaine-induced cardiac toxicity. These results are consistent with previous paediatric case reports demonstrating the ability of lipid emulsions to reverse cardiovascular collapse in this population. Although the primary outcome was not different between the three treatment groups, resuscitation with lipid emulsion alone induced fewer ECG abnormalities after ROSC compared with epinephrine alone or in combination with lipid emulsion.

It is usual practice to perform regional anaesthetic techniques under general anaesthesia in smaller children. General anaesthesia may mask cerebral toxicity, and therefore, the first manifestation of inadvertent intravascular injection of LA is more commonly cardiac arrhythmia or collapse. In contrast, seizure is often the first sign of LAST in adults (60% neurological symptoms as the first sign). Reported incidences of generalized tonic–clonic seizure during regional anaesthesia range from 0.2 to 0.9 per 1000 procedures performed in adults. The incidence of LAST during regional anaesthesia in neonates can be estimated using ECG changes or arrhythmias. Such changes were found in 0.5 per 1000 procedures performed in paediatric patients, including neonates.

Although LAST may be less frequent in neonates than in adults, it carries a higher risk of cardiac arrest. In view of this high risk, and differences in cardiac physiology and LA pharmacokinetics between newborn animals and adults, it is justifiable to perform further studies, evaluating different resuscitation strategies, incorporating the use of lipid emulsion and epinephrine.

**Lipid, epinephrine, or both**

Our results are consistent with those of Weinberg and colleagues that demonstrated that lipid emulsion alone improves survival in levobupivacaine-induced cardiac arrest in newborn piglets. Other recent animal studies, however, have advocated that vasopressin alone and in combination with epinephrine performed better than lipid emulsion in the treatment of bupivacaine toxicity. These discrepancies in the efficacy of lipid emulsions may reflect differences in study design. For example, in the study by Mauch and colleagues, cardiac massage was not used during resuscitation efforts, the injection of a lipid bolus was not followed by a continuous lipid infusion, epinephrine doses were different between study groups (3 vs 12 µg kg⁻¹ in the EPI + LIP group or 45 µg kg⁻¹ in the EPI group), and epinephrine was not combined with lipid emulsion during resuscitation. In the study by May and colleagues, piglets were rendered apnoeic, and therefore hypoxic, whereas hypoxia was avoided in our newborn pig model.

We found that epinephrine alone or in combination with lipid emulsion increased the number of ECG abnormalities recorded after ROSC. However, this did not have any effect on overall survival. In an experimental LAST study, performed by Harvey and colleagues, a single i.v. injection of 10 µg kg⁻¹ or greater of epinephrine was found to impair lipid-based resuscitation in rabbits. Epinephrine is widely used in neonatal resuscitation, but there is increasing evidence that high doses of

| Table 1 Baseline data. Mean (SD) |
|-----------------|--------|---------|--------|---------|
| (n)             | Age (range, days) | Weight (kg) | MAP (mm Hg) | HR (beats min⁻¹) |
| Control (n=7)   | 4–7    | 1.9 (0.3) | 60 (8)     | 122 (15)  |
| LIP (n=9)       | 6–9    | 2.5 (0.5) | 50 (15)    | 123 (4)   |
| Epinephrine (n=7)| 1–9   | 2.1 (0.7) | 61 (9)     | 137 (14)  |
| Lipid + epinephrine (n=10) | 5–7   | 1.9 (0.4) | 52 (7)     | 119 (13)  |

| Table 2 Duration of cardiopulmonary resuscitation in animals achieving ROSC |
|-----------------|---------|---------|---------|
| Control (n=1)   | Lipid (n=7) | Epinephrine (n=6) | Lipid + epinephrine (n=10) |
| Time (s)        | 720     | 460 (242)| 296 (167)| 304 (263) |
epinephrine may adversely affect patient outcomes.\(^{22}\) Compared with adults, the myocardium of the neonate exhibits a lower and less predictable positive inotropic response to adrenergic agonists such as epinephrine.\(^{17–28}\) Therefore, the effect of using epinephrine in neonates can be unpredictable compared with its use in adults. We also found that by infusing lipid emulsion, we significantly decreased the dose of epinephrine required. Although we did not study smaller doses of epinephrine than 10 \(\mu\)g kg\(^{-1}\), our results, combined with those from previous studies, support the idea that epinephrine doses in excess of 10 \(\mu\)g kg\(^{-1}\) may be more detrimental than beneficial in cardiac LAST in the newborn pig. Further research is needed to determine whether lipid alone or lipid combined with epinephrine at doses <10 \(\mu\)g kg\(^{-1}\) should be the first choice in this particular population.

**Levobupivacaine cardiac toxicity**

Reassuringly, the mean dose of levobupivacaine used to induce cardiovascular collapse in our newborn model was much higher than the maximal doses recommended during paediatric regional anaesthesia (2.5 mg kg\(^{-1}\)). Indeed, bupivacaine has been used to induce cardiac toxicity (asystole or a significant decrease in MAP) in the majority of animal studies to date, and the doses required in these various experimental models has always been higher than those used in anaesthesia. Our study confirmed that lipid emulsion is beneficial in cases of levobupivacaine cardiac toxicity, and that higher doses of levobupivacaine, compared with bupivacaine, may be needed to induce collapse in a newborn animal model of LAST.

**Limits of this study**

In the setting of LAST, the optimal time to administer lipid emulsion is a matter of some debate. Some authors recommend administering lipid emulsion when signs of central nervous system toxicity begin to appear, while others recommend waiting for signs of cardiac toxicity. Rosenblatt and colleagues\(^{2} \) performed cardiac resuscitation for \(\sim 30\) min before administering Intralipid\(^{\circledR}\). Warren and colleagues\(^{8}\) started lipid treatment within 10 min of CPR initiation. Litz and colleagues\(^{29}\) started lipid therapy within minutes of making the diagnosis of LA toxicity. However, regional anaesthesia in children is usually performed under general anaesthesia, and may mask signs of central nervous system toxicity. Therefore, cardiac arrhythmia or collapse may be the first presentation of cardiac toxicity in anaesthetized children; this is why we chose to treat with lipid emulsion at this late phase of LA toxicity.

It is difficult to compare our results with those of other similar studies, not only because of obvious differences in study design, but also because of variations in species used. Weinberg and Rubinstein\(^{30}\) have suggested that the pig may not be a suitable model for studying lipid resuscitation. Liposomes have been reported to cause a complement activation-related pseudoallergy (CARPA) affecting haemodynamic stability, and pigs have been documented as the worst models to observe this CARPA syndrome.\(^{31}\) The existence of CARPA reactions and the possible impact this would have on our results warrant further discussion. First of all, \(< 20\%\) of the complement system’s normal protective activity is present in newborn piglets. Therefore, the probability of CARPA occurring may be reduced in piglets. Secondly, we used lipid emulsion, as opposed to liposomes, and the potential to induce CARPA with lipid alone is still unknown. Thirdly, the lipid emulsion was infused only after LA intoxication. Finally, our results demonstrated better outcomes with lipid emulsion. Lipid emulsion was not associated with poorer survival rates; on the contrary, survival was improved compared with the control group. Crane and colleagues\(^{32}\) recently showed that 2 ml kg\(^{-1}\) of 20% lipid emulsion given i.v. more than 2–3 min, followed by a lipid emulsion infusion at 0.25 ml kg\(^{-1}\) min\(^{-1}\) for 20 min, did not induce CARPA in a

| Table 3 Number of ECG abnormalities in surviving piglets after ROSC. \(^{*}\)Statistically significant |
|-----------------|-----------------|-----------------|-----------------|
|                 | Lipid (n=7)     | Epinephrine (n=6) | Lipid + epinephrine (n=10) |
| Arrhythmia      | 0               | 11\(^{*}\)        | 10\(^{*}\)        |
| Conduction      | 0               | 3               | 7               |

| Table 4 Survival results after resuscitation per group and details of the Marascuilo procedure. \(^{*}\)Statistically significant |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Control (n=7)   | Lipid (n=9)     | Epinephrine (n=7) | Lipid + epinephrine (n=10) |
| Resuscitation per group |             |                 |                 |                 |
| Died            | 6               | 2               | 1               | 0               |
| Survived        | 1               | 7               | 6               | 10              |
| Absolute differences | 0.635         | 0.536           | –0.87 to –0.13\(^{*}\) |
| Critical range  | 0.714           | 0.523           | –0.92 to –0.18\(^{*}\) |
| 95% CI          | 0.857           | 0.370           | –0.97 to –0.46\(^{*}\) |
| Marascuilo procedure |             |                 |                 |                 |
| Control—lips    | 0.079           | 0.536           | –0.46 to 0.36   |
| Control—epinephrine | 0.222         | 0.387           | –0.10 to 0.55   |
| Control—lipids + epinephrine | 0.143       | 0.370           | –0.17 to 0.52   |
| Lipids—epinephrine | 0.079       | 0.536           | –0.46 to 0.36   |
| Lipids (lipids + epinephrine) | 0.222     | 0.387           | –0.10 to 0.55   |
pig model. The authors concluded that pigs could be used for lipid emulsion studies.

**Conclusion**

EPI, LIP, or EPI combined with LIP provided similar results for ROSC in a newborn pig model of levobupivacaine-induced cardiac toxicity. However, we found that epinephrine, alone or in combination with lipid, increased ECG abnormalities after ROSC in this population.

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**Declaration of interest**

None declared.

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