Lipid Emulsion Combined with Epinephrine and Vasopressin Does Not Improve Survival in a Swine Model of Bupivacaine-induced Cardiac Arrest

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Background: This study sought to evaluate the efficacy of lipid emulsion in reversing bupivacaine-induced cardiovascular collapse when added to a resuscitation protocol that included the use of epinephrine and vasopressin.

Methods: After induction of general anesthesia and instrumentation, 19 mixed-breed domestic swine had cardiovascular collapse induced by an intravenous bolus of 10 mg/kg bupivacaine. After 5 min of resuscitation including chest compressions, epinephrine (100 μg/kg) and vasopressin (1.5 U/kg), animals were randomized to receive either a bolus of 20% lipid emulsion (4 ml/kg) followed by a continuous infusion (0.5 ml · kg^{-1} · min^{-1}) or an equal volume of saline. Investigators were blinded to the treatment assignment. The primary endpoint was return of spontaneous circulation (mean arterial pressure of at least 60 mmHg for at least 1 min).

Results: Treatment groups were similar with respect to baseline measurements of weight, sex, and hemodynamic and metabolic variables. The rates of return of spontaneous circulation were similar between groups: (3 of 10) in the lipid group and 4 of 9 in the saline group ($P = 0.65$). Total serum bupivacaine concentrations were higher in the lipid group at the 10-min timepoint (mean ± SEM: 23.13 ± 5.37 ng/ml *vs.* 15.33 ± 4.04 ng/ml, $P = 0.004$). More norepinephrine was required in the lipid group compared to the saline group to maintain a mean arterial pressure above 60 mmHg during the 60-min survival period (mean ± SEM: 738.6 ± 94.4 *vs.*. 487.3 ± 171.0 μg).

Conclusions: In this swine model, lipid emulsion did not improve rates of return of spontaneous circulation after bupivacaine-induced cardiovascular collapse.

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SYSTEMIC toxicity from local anesthetic overdose can occur from accidental intravascular injection, drug overdose, or rapid absorption from the administration site. The risk of toxicity is low when appropriate dosing and injection are used; the incidence is estimated at 7.5 to 20 events per 100,000 in adults.[1] Toxicity manifests in the central nervous system as confusion, seizure, and coma and in the cardiovascular system as dysrhythmia and eventually cardiac arrest. Treatment of cardiovascular collapse caused by local anesthetic overdose in humans is notoriously difficult to treat.[2-4]

There are conflicting reports in the literature regarding the efficacy of lipid emulsion in the treatment of cardiac arrest caused by local anesthetic toxicity. Rodent and canine models in which cardiac arrest was induced with a rapid bolus of intravenous bupivacaine report 100% survival with the administration of lipid emulsion and 100% mortality without lipid emulsion; no vasopressors were used in either group.[5-8] These results contrast with the findings in a swine model used by Mayr *et al.*[9,10] in which 100% of swine were resuscitated from a cardiac arrest induced by an intravenous bolus of 5 mg/kg bupivacaine after treatment with chest compressions and vasopressors. When these authors[9] examined the use of lipid emulsion without the use of vasopressors in their model, they found that none of the animals could be resuscitated.

Several case reports suggest that lipid emulsion may be beneficial in the treatment of cardiac arrest associated with local anesthetic toxicity.[11-14] These case reports describe the administration of lipid emulsion in conjunction with the use of vasopressors. Other case reports[15,16] describe the use of standard resuscitation measures without the use of lipid emulsion in the successful resuscitation of patients with presumed ropivacaine-induced cardiac arrest. These cases raise the question of whether lipid emulsion truly accounts for the successful resuscitation.

In view of the conflicting findings from laboratory investigations and the limited evidence in humans, further evidence is required to confirm the benefit of adding lipid emulsion during resuscitation from cardiac arrest induced by local anesthetic. We tested the hypothesis that return of spontaneous circulation would be more frequent after bupivacaine-induced cardiac arrest when lipid emulsion was added to a resuscitation protocol that includes epinephrine and vasopressin.

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Materials and Methods

Study Design

We conducted a randomized, controlled laboratory experiment. The University of Pittsburgh Institutional Animal Care and Use Committee (Pittsburgh, Pennsylvania) approved this investigation (protocol number 0504395). We used 20 mixed-breed domestic swine of either sex, ranging in weight from 23.2 to 27.3 kg (mean = 25.7 kg). We assigned animals to one of two experimental treatments by using a computer-generated randomization sequence. Before enrollment, technical difficulties were encountered with one animal, leaving us with 10 animals in the lipid-treated group and 9 animals in the placebo group. Investigators who performed the animal instrumentation and resuscitation were blinded to treatment groups by having a separate investigator prepare and administer the experimental treatments in opaque and covered syringes. Figure 1 outlines the time sequence of the experimental protocol.

Premedication and Induction of Anesthesia

The animals were sedated with 10 mg/kg intramuscular ketamine and 4 mg/kg xylazine (α-2 adrenergic agonist). This combination of sedatives is widely used in animal surgery because of its hemodynamic stability.[17] Intravenous access was established *via* a peripheral ear vein by using a 20-gauge catheter. A surgical plane of anesthesia was achieved by using a rapid intravenous infusion of α-chloralose (40 mg/kg) and maintained with a continuous infusion of the same ($10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$).
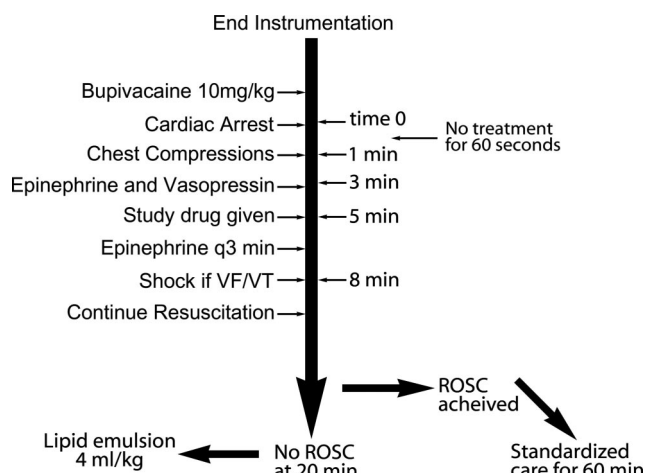

Fig. 1. Timeline of experimental protocol. Initial vasopressors were 100 μg/kg epinephrine and 1.5 U/kg vasopressin administered 3 min after confirmation of cardiac arrest; 15 μg/kg epinephrine was repeated every 3 min after the first dose until return of spontaneous circulation (ROSC) was achieved. Electrical defibrillation was given when appropriate at a fixed biphasic dose of 150 joules. VF = ventricular fibrillation; VT = ventricular tachycardia.

Animal Instrumentation

Tracheal intubation for each animal was achieved by direct laryngoscopy, inserting a size 5.0 cuffed endotracheal tube. The swine were ventilated with room air by using a volume-cycled ventilator (Ohmeda 7000, GE Healthcare, Madison, WI). Ventilation was initiated at a tidal volume of 12–14 ml/kg, a ventilatory rate of 12–16 breaths/min, and an inspiration:expiration ratio of 0.40. Ventilation was adjusted to maintain eucapnea (end-tidal carbon dioxide 35–45 mmHg). This was measured with a mainstream capnometer (Zoll M Series; CCT, Chelmsford, MA) that is incorporated into the defibrillator monitor. Core body temperature was measured by placing a nasopharyngeal probe (Bi-Temp Temperature Monitor; Respiratory Supply Products, Inc., Irvine, CA) approximately 10 cm into the animals' esophagus. The animals' forelimbs were shaved, and three surface electrodes were secured and configured to correspond to a standard Lead II electrocardiogram. The electrocardiogram data were monitored and recorded continuously. The unfiltered electrocardiogram signal is passed through a wide band-pass preamplifier with a 10-fold direct current gain and digitized at 1000 samples/s (MacLab, ADInstruments, Castle Hill, Australia).

After the airway was secured and surgical depth of anesthesia was established, neuromuscular paralysis was achieved with pancuronium (4-mg initial intravenous bolus with additional 2-mg boluses as needed). A direct cutdown was used to cannulate the femoral artery and vein with 9-French catheters. Continuous central pressures were obtained with transducers placed into the ascending aorta and to the right atrium (Mikro-Tip transducer models SPC 471A and SPC 3705; Millar Instruments, Houston, TX). These were used to calculate coronary perfusion pressure during chest compressions, defined as aortic diastolic pressure minus right atrial pressure, as well as continuously throughout the postresuscitation period. These data were acquired digitally at a sampling rate of 1000 points/s with a commercially available software package (Chart, v.3.5/s, DInstruments). Arterial blood gas was measured as soon as arterial access was established (Portable Clinical Analyzer, I-Stat; Heska Corp., Waukesha, WI). Arterial blood gas was measured upon obtaining arterial access, before the induction of cardiac arrest, and throughout the resuscitation and postresuscitation periods. The anesthesia time was recorded, which we defined as the time from when the initial bolus of alpha choralose was given until the time cardiac arrest was induced with bupivacaine. Standardization of this time interval was achieved by initiating cardiac arrest as closely as possible to 35 min after induction of anesthesia.

Induction of Cardiovascular Collapse

Under general anesthesia, cardiovascular collapse was induced with an injection of 10 mg/kg bupivacaine

(0.75%, preservative-free) given over 10 s through a central venous catheter into the right atrium. Cardiovascular collapse was defined on the electrocardiogram as asystole, ventricular fibrillation, or pulseless electrical activity, and confirmed with a mean arterial pressure of less than 30 mmHg persisting for 15 s, corresponding to time 0 (fig. 1). After confirming cardiac arrest, standard resuscitation protocols were implemented in both experimental groups at 1 min, including external chest compressions at an uninterrupted rate of 100 per minute and depth of 5 cm in the anteroposterior direction with a pneumatic compression device (LUCAS CPR®; Jolife, Lund, Sweden). Positive pressure ventilations were manually delivered with a self-inflating bag using 100% oxygen at a rate of 10 breaths/min. Intravenous injection of epinephrine (100 μg/kg) and vasopressin (1.5 u/kg) were given at 3 min after cardiac arrest, and defibrillation of appropriate rhythms was initiated at 8 min after cardiac arrest. Compressions were paused 10 s every 50 s (last 10 s of each minute) to examine the characteristics of the underlying electrocardiogram rhythm and to assess the need for defibrillation (biphasic 150 Joules). Epinephrine (15 μg/kg) was administered every 3 min after the first dose for the duration of the resuscitation until return of spontaneous circulation (ROSC).

Intervention Medication and Outcomes

After 5 min of a standardized resuscitation protocol, animals were randomized to receive either a bolus (4 ml/kg) over 2 min of 20% lipid emulsion (Intralipid®; Baxter, Deerfield, IL) followed by a continuous infusion $(0.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$ for the next 10 min or an equal volume of saline (4 ml/kg) as bolus and continuous infusion. The primary outcome, ROSC, was defined as an organized rhythm on the electrocardiogram with a cardiac output that supported a mean arterial pressure of at least 60 mmHg for at least 60 s. Similar endpoints have been used in this swine model of cardiac arrest many times, in electrically induced cardiac arrest,[18,19] and in local anesthetic–induced cardiac arrest.[9,10,20] In animals that achieved ROSC, norepinephrine was administered to maintain a mean arterial pressure of at least 60 mmHg and titrated to keep the mean arterial pressure between 60 and 85 mmHg. In addition, arterial blood gas analysis was used to compare the level of metabolic insult between groups during the survival phase. If ROSC was not achieved after 20 min, some of the animals were given a bolus of 4 ml/kg of lipid emulsion, and resuscitation was continued for another 10 min with chest compressions and 15 μg/kg epinephrine was administered every 3 min.

Sample Collection and Preparation

Blood samples were obtained at 3, 10, 17, 30, and 60 min after cardiac arrest for the measurement of total bupivacaine concentrations. Blood was withdrawn from the central venous catheter, allowed to clot, and centri-fuged, and the serum was collected and stored at −80°C. Serum was subsequently thawed, and 2 ml of 0.1% formic acid in deionized water containing 50 ng/ml ropivacaine (internal standard) was added to 50 μl of serum. All glass test tubes were silanized. Vortexed samples were extracted using 60-mg hydrophilic lipophilac balance (HLB) solid phase extraction cartridges (Oasis; Waters, Milford, MA). Columns were washed with one 3-ml volume of 5% methanol and were eluted with 100% methanol. Extracts were dried under nitrogen gas at 37°C and reconstituted in 1 ml of 95:5 0.1% formic acid:acetonitrile. Blank and spiked serum samples with and without exogenous 20% lipid emulsion (Intralipid®) up to 30% v/v were prepared as above with no added drug and drug standards at varying concentrations, respectively, for assay validation.

High Performance Liquid Chromatography Analysis

Serum concentrations were determined by reversed-phase high-performance liquid chromatography with mass spectrometric detection. The system consisted of a Surveyor autosampler and pump and a single quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA). Separations were achieved with a Betabasic C-18, 5 μm (150 × 2.1 mm) reversed-phase column (ThermoHypersil®, Bellefont, PA) under ambient temperature at a flow rate of 0.2 ml/min. Serum extracts were separated by using a gradient elution starting at 0.1% formic acid in deionized water (A) and acetontrile (B) at a mixture of 95:5. Mobile phase B increased from 5% to 95% in a linear gradient over 12 min and returned to initial conditions over 1 min with a 3-min preequilibration period before the next sample run. Twenty microliters were injected, and analysis was conducted with a probe temperature and voltage of 350°C and 4.0 kV, respectively. Cone voltage was set at 50 V, and selective ion monitoring in positive electrospray ionization mode was carried out for specific m/z 288.8 (bupivacaine) and m/z 274.93 (ropivicaine). The retention times for bupivacaine and ropivacaine were 6.75 min and 6.25 min, respectively.

Sample Size Requirements

Estimates of effect size for ROSC were based on the canine experiment by Weinberg *et al.*[7,8] The canine data demonstrated an effect size in the placebo group of 0% survival and 100% survival in the treatment group, with 10 mg/kg bupivacaine administered to induce cardiac arrest. Previous experiments in the swine model by Mayr *et al.*[9,10] used a smaller dose of bupivacaine (5 mg/kg), which resulted in 100% survival in the animals treated with epinephrine and vasopressin. Considering the higher dose of bupivacaine in the current experiment, we predicted it would be more difficult to achieve 100% ROSC in the treatment group; we therefore estimated a survival of 75% in the lipid group and 5% survival in the

placebo group at a dose of 10 mg/kg bupivacaine. Sample size calculations showed that a Fisher exact test with a 0.050 two-sided significance level will have 86% power to detect a difference between the lipid group survival of 75% and placebo group survival of 5% when the sample size in each group is 9. To account for potential attrition, we enrolled 10 animals per group.

Statistical Interpretation

Baseline data consisting of measurements made before the induction of cardiac arrest, including anesthesia time (minutes), weight (kg), arterial blood gas, heart rate, and mean arterial pressure, were compared between groups by using two-sided Student t test. For the primary outcome of ROSC, the number of animals achieving ROSC was compared with Fisher exact test. Time to ROSC after lipid injection (minutes) was compared between groups using Kaplan-Meier survival curves (Log-rank test). Measurements of coronary perfusion pressures were analyzed using repeated measures ANOVA. Descriptive statistics (mean ± SD) are presented for hemodynamic and acid-base data for those animals achieving ROSC. The concentration of total serum bupivacaine measured during the 20 min of cardiopulmonary resuscitation were compared between groups using repeated measures analysis of variance, with time and group as factors (α = 0.05). Independent samples T test were used for *post hoc* analysis with a Bonferroni correction (α = 0.016). Where appropriate, normality of data distribution was evaluated using box-plots and comparison of mean and medians. Levene's test was used to assess homogeneity of variances (SPSS version 16; SPSS Inc., Chicago, IL).

Results

Treatment groups were similar with respect to baseline measurements of weight, sex, and hemodynamic and metabolic variables (tables 1, 2, and 3). Baseline measurements of heart rate (table 2), however, were higher in the saline group (mean ± SD: 124.1 ± 20.4 *vs.* 107.6 ± 9.7, P = 0.009). The time to cardiovascular collapse after bupivacaine injection was similar between groups (mean ± SD: 39 ± 6.7 s in the lipid group and 38.7 ± 7.5 s in the saline group, P = 0.87). Analysis of coronary perfusion pressures (fig. 2) demonstrated a significant main effect of time (P = 0.002), however, main effect of group was not significant (P = 0.46), and there was no interaction between group and time (P = 0.66). The total number of shocks administered was similar between groups (mean ± SE: 8.0 ± 2.1 in the lipid group and 8.9 ± 2.1 in the saline group, P = 0.77).

Primary Outcomes

There was no difference in rates of ROSC between groups, with 3 of 10 (30%) in the lipid group and 4 of 9

Table 1. Baseline Characteristic and Resuscitation Data

	Saline (n = 9)	Lipid (n = 10)
Weight, kg (mean ± SD)	25.8 ± 1.29	25.7 ± 1.33
Sex, % male	44%	60%
Anesthesia time, min (mean ± SD)	34.9 ± 6.9	32.1 ± 5.4
Time to CA, s (mean ± SD)	38.7 ± 7.5	39.2 ± 6.7
Number that achieved ROSC by 20 minutes	4 of 9	3 of 10
Time to ROSC, min (median ± 95% CI)	8.5 (4.6–12.4)	9.0 (7.4–10.6)
Number of shocks (mean ± SE)	8.89 ± 2.1	8.0 ± 2.1
Norepinephrine, µg (mean ± SE)	487.3 ± 94.4* (n = 4)	738.6 ± 171.0 (n = 3)

Anesthesia time is defined as the time from induction of anesthesia until the administration of bupivacaine. Norepinephrine is presented as the total cumulative amount used during the 60 min after ROSC (n = 7).

* More norepinephrine was required in the lipid group to maintain mean arterial pressure above 60 mmHg in the animals that achieved ROSC (P = 0.034).

CA = cardiac arrest; ROSC = return of spontaneous circulation.

(44%) in the saline group achieving ROSC (Fisher exact test, P = 0.65). In those animals achieving ROSC (n = 7), there was no difference in time when ROSC occurred (log-rank test, P = 0.52); median ROSC time was 9.0 min (95% CI 7.4–10.6) in the lipid group and 8.5 min (95% CI 4.6–12.4) in the saline group. Animals that achieved ROSC in both groups received 130 to 145 µg/kg epinephrine during resuscitation (initial 100 µg/kg followed by 2 or 3 more doses of 15 µg/kg, depending on when ROSC occurred).

Secondary Outcomes

All animals that achieved ROSC survived to the 1-hour endpoint (n = 7). Dysrhythmias were noted during this period in both groups; for example, wide QRS complex, premature ventricular contractions, and premature atrial contractions (See video, Supplemental Digital Content 1, which shows a real-time physiologic tracing from a representative animal undergoing the experimental protocol; tracings from top to bottom: aortic pressure, central venous pressure, calculated coronary perfusion pressure, and the electrocardiogram, http://links.lww.com/A1198). The amount of norepinephrine administered over the 60 min after ROSC was higher in the lipid group (mean ± SEM: 738.6 ± 94.4 *vs.* 487.3 ± 171.0 µg, P = 0.034). Norepinephrine was required in all seven animals that achieved ROSC to maintain mean arterial pressure of at least 60 mmHg. The infusion rate of norepinephrine ranged from 1–15 µg/min and varied in accordance to the target mean arterial pressure. Hemodynamic (table 2) and metabolic variables (table 3) were similar in the two groups during the first hour after ROSC. In those animals that

Table 2. Hemodynamic Data

	Baseline (n = 19)	Time after ROSC n = 7			
		15 min	30 min	45 min	60 min
HR, min^{-1}					
Saline	124.1 ± 20.4	153.4 ± 14.9	148.2 ± 30.9	140.6 ± 24.8	154.4 ± 43.9
Lipid	107.6 ± 9.7*	128.9 ± 7.8	127.8 ± 35.5	196.9 ± 88.7	205.7 ± 88.2
MAP, mmHg					
Saline	84.6 ± 8.9	110.1 ± 12.2	86.1 ± 13.4	89.3 ± 14.4	73.4 ± 33.7
Lipid	82.9 ± 12.2	83.9 ± 10.4	83.6 ± 8.9	80.2 ± 13.7	69.5 ± 7.8
CVP, mmHg					
Saline	6.6 ± 1.2	8.9 ± 2.6	8.0 ± 3.4	7.2 ± 4.3	8.3 ± 3.7
Lipid	6.5 ± 1.5	9.5 ± 2.1	8.6 ± 2.2	8.3 ± 2.7	8.3 ± 2.1

Results are shown as mean ± SD. Baseline data consist of measurements taken before the administration of bupivacaine. Differences between groups were tested with independent sample *t* test at baseline.

* Heart rate was higher in the saline group at baseline (*P* = 0.009). Timepoints after ROSC (15, 30, 45, and 60 minutes) are measured after achieving ROSC (n = 4 in the saline group and n = 3 in the lipid group).

CVP = central venous pressure; HR = heart rate; MAP = mean arterial pressure; ROSC = return of spontaneous circulation.

did not achieve ROSC by the primary endpoint, the administration of lipid emulsion to 3 animals in the saline group and 7 in the lipid group did not lead to any ROSC when resuscitation was continued for 10 min past the primary endpoint.

The high performance liquid chromatography (mass spectrometric) assay used for assessment of total bupiva-

caine serum concentrations was validated before use. The peak area ratio *versus* drug concentration was weighted by the reciprocal of the peak area ratio and was linear over the range of 0.01–2 μg/ml (R^2 > 0.99), corresponding to 0.2–40 μg/ml in the serum before dilution. Interday coefficients of variation were 3.06% and 2.95% at 0.1 and 1 μg/ml bupivacaine in the diluted samples, respectively, and

Table 3. Metabolic Data

	Baseline (n = 19)	Time after ROSC (n = 7)			
		15 min	30 min	45 min	60 min
pH					
Saline	7.45 ± 0.05	7.25 ± 0.08	7.24 ± 0.02	7.30 ± 0.04	7.38 ± 0.05
Lipid	7.46 ± 0.04	7.15 ± 0.03	7.14 ± 0.04	7.24 ± 0.09	7.32 ± 0.07
pco$_2$, mmHg					
Saline	37.7 ± 2.6	46.8 ± 7.7	48.5 ± 6.4	45.9 ± 4.5	39.5 ± 2.8
Lipid	37.2 ± 4.1	61.6 ± 3.8	61.2 ± 6.6	44.1 ± 3.4	41.7 ± 2.1
po$_2$, mmHg					
Saline	85.7 ± 34.5	308.8 ± 121.6	234.3 ± 81.3	270.75 ± 57.3	234.8 ± 63.3
Lipid	101.7 ± 32.8	213.7 ± 192.8	279.7 ± 201.2	181.7 ± 139.8	205.0 ± 177.5
HCO$_3$, mmol/l					
Saline	25.6 ± 3.6	20.47 ± 2.8	20.8 ± 2.8	22.6 ± 2.9	23.2 ± 3.0
Lipid	26.5 ± 2.4	21.6 ± 1.8	21.1 ± 3.8	19.3 ± 4.7	21.7 ± 3.3
BE, mmol/l					
Saline	1.55 ± 4.19	−6.75 ± 3.95	−6.50 ± 2.51	−3.75 ± 3.30	−1.75 ± 3.95
Lipid	2.70 ± 2.79	−7.33 ± 1.52	−8.33 ± 4.51	−8.00 ± 6.08	−4.00 ± 4.58
Hct, fraction					
Saline	22.2 ± 8.7	28.5 ± 5.1	35.5 ± 12.4	35.0 ± 7.1	29.75 ± 4.6
Lipid	25.9 ± 2.3	27.0 ± 4.6	26.3 ± 7.1	26.7 ± 2.1	26.3 ± 5.1
Glucose, mg/dL					
Saline	107.5 ± 25.6	143.5 ± 11.6	111.0 ± 28.6	100.0 ± 36.8	96.8 ± 39.3
Lipid	106.9 ± 22.4	152.7 ± 67.7	140.3 ± 74.6	116.0 ± 62.6	118.7 ± 47.8
K, mmol/l					
Saline	3.5 ± 0.7	4.22 ± 0.6	3.28 ± 0.2	3.3 ± 0.2	3.6 ± 0.3
Lipid	3.7 ± 0.3	4.6 ± 1.7	3.58 ± 0.5	3.7 ± 0.4	4.1 ± 0.3
Na, mmol/l					
Saline	142.0 ± 1.5	143.0 ± 2.7	143.8 ± 1.9	143.3 ± 2.2	142.0 ± 2.2
Lipid	142.1 ± 2.18	138.7 ± 3.8	138.7 ± 3.5	139.7 ± 5.1	137.0 ± 3.5

Results are shown as mean ± SD. Baseline data consist of measurements taken before the administration of bupivacaine. Differences between groups were tested with independent sample *t* test at baseline. Groups did not differ at baseline on any variable. Time points after return of spontaneous circulation (ROSC) (15, 30, 45, and 60 minutes) are measured after achieving ROSC (n = 4 in the saline group and n = 3 in the lipid group).

BE = base excess; Hct = hematocrit; pco$_2$ = partial pressure of carbon dioxide; po$_2$ = partial pressure of oxygen.
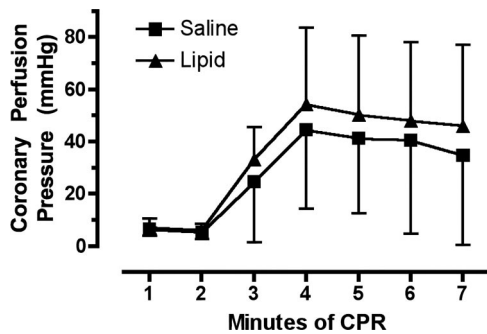
Fig. 2. Coronary perfusion pressure during cardiopulmonary resuscitation. Coronary perfusion pressure is calculated by subtracting the measured aortic diastolic pressure from the measured right atrial pressure during chest compressions. Data presented as mean ± SD. Repeated measures analysis of variance revealed a significant main effect of time ($P = 0.002$). CPR = cardiopulmonary resuscitation.

were not affected by the addition of up to 30% v/v of 20% lipid emulsion. Similarly, drug recoveries from spiked serum samples were not affected by the absence or presence of 30% v/v of 20% lipid emulsion and were 95.16 ± 3.77 and 98.2 ± 7.6%, respectively.

Analysis of total bupivacaine concentrations revealed a significant main effect of time ($P = 0.0001$) (fig. 3). Interaction between time and group was not significant ($P = 0.06$). There was a significant main effect of group ($P = 0.004$). Post hoc analysis using independent samples t test and a Bonferroni correction revealed that bupivacaine concentrations were similar between groups before treatment assignment at 3 min ($P = 0.87$) and similar at 17 min ($P = 0.5$). Bupivacaine concentrations were greater in the lipid group compared to the saline group at 10 min (mean ± SD: 23.13 ± 4.04 ng/ml vs. 15.33 ± 5.37 ng/ml, $P = 0.004$).
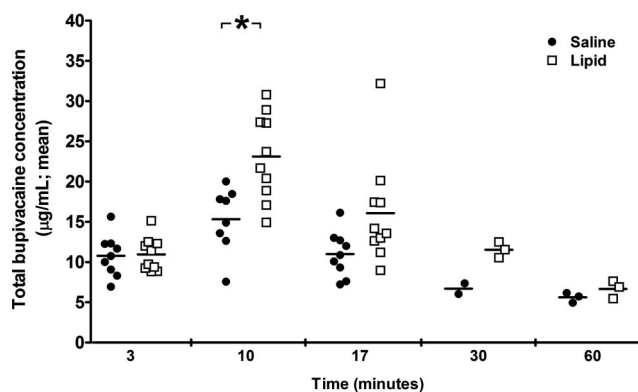

Fig. 3. Total serum bupivacaine concentrations measured with high-performance liquid chromatography. Measurements at the 30-min and 60-min timepoints only include data from animals that achieved return of spontaneous circulation after 20 min of resuscitation (n = 7). Repeated measures analysis of variance comparing the 3-, 10-, and 17-min timepoints demonstrated an effect of time ($P = 0.001$) and an effect of group ($P = 0.004$). * Post hoc analysis revealed that bupivacaine concentrations were significantly higher in the lipid group at 10 min ($P = 0.009$).

Discussion

Using a swine model of bupivacaine-induced cardiac arrest, we observed that lipid emulsion did not improve ROSC when added to a resuscitation protocol that included epinephrine and vasopressin. These results contrast with those reported in a rodent[6] and canine models[7,8] in which vasopressors were not used. Our results are consistent with and extend the findings of Mayr et al.[9] in a similar swine model that examined vasopressors (without lipid) compared to lipid (without vasopressors). Differences between these experimental models and interspecies differences may account for the conflicting results.

Several authors have demonstrated the advantages of the swine model over other species based on the similarities of the pig heart to that of human.[21-24] White et al.[23] demonstrated that the dog heart has an extensive collateral coronary circulation four times more developed than that of the pig and human. They also identified that coronary blood flow in the dog is substantially greater than that of the pig. Exercise capacity differs greatly between species; the maximal oxygen consumption in the dog is 82 ml · kg^{-1} · min^{-1}, almost twice that of the swine. These differences translated into a survival benefit with a smaller infarct size and decreased mortality in the dog compared to the pig when subjected to occlusion of the left anterior descending artery. Swine are more prone to lethal arrhythmias than the rat and the dog in models of acute coronary artery occlusion.[24] Further supporting the model selection, Weaver et al.[25] found that the coronary anatomy and distribution of coronary blood supply have remarkable similarities in human and swine hearts.

An important strength in the experimental protocol in our study was the type of chest compressions that closely approximate advanced cardiac life support guidelines. We used closed chest compressions, in contrast to open chest cardiac massage used in the dog model. Mean arterial pressure, cardiac output, and coronary perfusion are greater with the use of the open-chest technique compared to closed-chest cardiopulmonary resuscitation. Open-chest massage produces a cardiac output averaging 67% of controls, compared with 15% of controls using closed-chest massage.[26,27] Other studies have demonstrated an improved resuscitation rate in a ventricular fibrillation model[28,29] with the open-chest compared to the closed-chest method of resuscitation. With improved cardiac output during resuscitation, the administration of intravenous medications may have a falsely decreased circulation time compared to closed-chest cardiopulmonary resuscitation.

Comparing our findings with observations from the canine model must take into consideration not only interspecies differences and the type cardiopulmonary resuscitation used, but also the use of vasopressors in the

control group. The fundamental importance of generating adequate coronary perfusion pressure during cardiopulmonary resuscitation has been demonstrated in animal models and human trials.[30–34] Coronary perfusion pressure correlates with myocardial blood flow during chest compressions and likewise correlates with rates of return of spontaneous circulation.[30] This concept is important when considering how or if lipid emulsion improves resuscitation in any given model of cardiac arrest. Lipids are known to increase both mean arterial pressure and systemic vascular resistance.[35–39] Grekin *et al.*[38] demonstrated in a rat model that an alpha antagonist obliterates the elevation in mean arterial blood pressure caused by lipid infusion. Therefore, an alternative explanation to the "lipid sink" hypothesis[5] could be that lipids have hemodynamic effects in rodent and canine models that improve coronary perfusion pressure during cardiopulmonary resuscitation to reach the critical threshold required to achieve ROSC.

The use of vasopressors in the treatment of cardiac arrest caused by local anesthetic overdose is controversial. Using a rat model of bupivacaine cardiac arrest, Weinberg *et al.*[6] concluded that lipid emulsion (without vasopressors) improved recovery compared to epinephrine. Cardiac arrest was induced with 20 mg/kg bupivacaine, and rats were randomized into one of three groups: lipid, epinephrine, or saline (n = 5 per group). Primary endpoints for determining improved recovery were defined as the RPP, acid base status, and QRS duration 10 min after the infusion of bupivacaine. The RPP, acid base status, and QRS duration were better in the lipid group compared to the epinephrine group. All animals achieved ROSC (an unassisted arterial pulse) in the 10-min experiment. An alternative conclusion is mentioned in their discussion; more rats actually recovered in the epinephrine group, if recovery is defined as mean arterial blood pressure greater than 80 mmHg for more than 5 min (3 of 5 in the epinephrine group compared to 2 of 5 in the lipid group). Interpreting these results is difficult considering that all of the saline-treated rats achieved spontaneous circulation, whereas saline alone yields no survivors in both the canine and swine models.

The dose of vasopressor used in our experiment was significantly larger than those used in clinical practice (for example, in a 70-kg person, 1 mg of epinephrine would be 15 μg/kg and 40 U of vasopressin would be 0.6 U/kg). We used an initial dose of epinephrine (100 μg/kg) combined with vasopressin (1.5 U/kg) given 3 min after cardiac arrest; epinephrine (15 μg/kg) was then repeated every 3 min until ROSC was achieved or until study termination. Mayr *et al.*[9,10] used 45 μg/kg epinephrine and 0.8 U/kg vasopressin given at 5 and 10 min after cardiac arrest followed by 200 μg/kg and 0.8 U/kg at 15 min, respectively. Lindner *et al.*[40] established the optimal dose of epinephrine in the swine model; they examined four doses of epinephrine 15, 30, 45, and 90 μg/kg given 4 min after ventricular fibrillation. They found that coronary perfusion pressure, left ventricular myocardial blood flow, and ROSC rates were higher in the 45 and 90 μg/kg groups compared to the two lower-dose groups. Further studies established that the combination of epinephrine and vasopressin was superior in the swine model of ventricular fibrillation,[41,42] pulseless electrical activity,[43] and asphyxial cardiac arrest,[44–46] and recently in the swine model of bupivacaine-induced cardiac arrest.[9]

Although the dose of vasopressor in our study is based on several swine experiments advocating the use of vasopressors in several types of cardiac arrest, our study does not rule out a potential detrimental interaction between lipid emulsion and the administration of epinephrine and vasopressin specific to the setting of bupivacaine cardiac arrest. The use of relatively high doses of epinephrine and vasopressin before the lipid emulsion treatment in our model could potentially have deleterious effects on the possible benefits of lipid emulsion in this setting. Further studies are required to assess lipid infusion added to lower doses of vasopressors in this particular model. We did not include a group treated with lipid only because none of the animals could be resuscitated in the placebo groups of the canine and swine models. Furthermore, we predicted it would be highly unlikely that any animal could achieve ROSC with lipid alone in our experimental protocol, given that twice the dose of bupivacaine was used in our study compared to the 5 mg/kg in the Mayr *et al.*[9,10] studies. This decision is further supported by the coronary perfusion pressure tracings provided in these reports, where neither the saline-treated group[10] nor the lipid-treated group[9] achieved the critical threshold of 20–30 mmHg, and none of these animals achieved ROSC.

Our experimental protocol differed from that of Mayr *et al.*[9] who used hypoxia to simulate tonic clonic seizure. Their protocol included stopping ventilation from the time of bupivacaine injection (5 mg/kg) until the onset of asystole (3 ± 1 min) to simulate seizure activity. Our study did not introduce apnea at this stage, and cardiac arrest ensued in less than 1 min after bupivacaine injection (10 mg/kg). Heavner *et al.*[47] demonstrated that severe hypoxia decreased the threshold of bupivacaine to cause seizures and cardiac dysrhythmias in swine.

Our study has several limitations, including the use of young and healthy animals. One common theme in case reports of local anesthetic toxicity is that many of these patients have significant comorbidities such as coronary artery disease, diabetes mellitus, and advanced age. These factors alter the pharmacology of local anesthetics and rates of resuscitation from cardiac arrest. Younger animals are more resistant to the toxic effects of bupivacaine.[48] The use of anesthetic medications in these animal models also limits their applicability to clinical practice. For example, propofol would clearly be a con-

founding variable in these experiments.[49] Accordingly, we chose α-chloralose, which is similar to pentathol and has minimal cardiovascular effects in swine.[50] However, a potential interaction between this medication and lipid in this model cannot be excluded. Another limitation of our study relates to a modest difference in the baseline heart rate between groups before induction of cardiac arrest (table 2). One possible explanation is that the difference in baseline heart rate was due to variable timing of pancuronium administration during the instrumentation period. It is uncertain that these small differences in heart rate at baseline would have had any important influence on the primary outcome of this experiment. Furthermore, our results are limited by a small sample size. The power analysis used in designing this study was based on a large effect size between groups that was not demonstrated in our model.

There is much variability in a particular species susceptibility to local anesthetic toxicity. This susceptibility may be caused by differences in the volume of distribution and half-life of bupivacaine between the dog[51] and swine models.[52] Swine seem to be more susceptible than dogs to the toxic effects of bupivacaine. A bolus dose of 10 mg/kg bupivacaine in the canine model[7,8] produced cardiac collapse within 7 min, compared to less than 1 min in our swine model. Using a dog model, Kasten *et al.*[53] demonstrated that successful resuscitation from cardiac arrest was possible after 76.8 ± 20.4 mg/kg bupivacaine by using epinephrine and open chest cardiac massage. In contrast to the dog model, ROSC is not possible without vasopressors when cardiac arrest is induced with only 5 mg/kg of bupivacaine in the swine model.

The lipid sink theory[5] proposes that the lipid emulsion binds free bupivacaine in plasma, and it would therefore be expected that higher total bupivacaine concentrations would be observed in the lipid-treated group. We found that total bupivacaine concentrations were higher only at the 10-min point (fig. 3). Despite this difference, there was no effect on ROSC or coronary perfusion pressures at any point in the experiment. The bupivacaine concentrations achieved were higher in our experiment relative to the results reported by Mayr *et al.* because of the increased dose of bupivacaine administered (10 mg/kg *vs.* 5 mg/kg, respectively). Only the total concentrations of bupivacaine were measured in lieu of free drug concentrations because of concerns regarding the effects of freezing and thawing the blood. Future studies should examine free (non–lipid-bound) concentrations of bupivacaine from fresh samples to quantify the presumed pharmacologically active fraction.

In a swine model, lipid emulsion did not improve ROSC from bupivacaine-induced cardiac arrest when added to a resuscitation protocol that included the use of epinephrine and vasopressin. We have highlighted the differences among the various models and species used

in these studies, such as the cardiac anatomy and physiology of the animal under investigation, the susceptibility of a particular species to local anesthetic toxicity, the type of cardiopulmonary resuscitation used in the model, whether or not vasopressors are used, and the extent of hypoxia initially experienced by the animals. These differences could account for the inconsistent results obtained in different experimental models. Further studies are needed to confirm the benefit of lipid emulsion in resuscitation from bupivacaine-induced cardiac arrest.

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