Reversal of Bupivacaine-Induced Cardiac Electrophysiologic Changes by Two Lipid Emulsions in Anesthetized and Mechanically Ventilated Piglets

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BACKGROUND: Accidental IV administration of bupivacaine can compromise cardiovascular function by inducing lethal arrhythmias whose hemodynamic consequences may be alleviated by lipid emulsions. However, little is known about the electrophysiologic effects of lipid emulsions. In this study, we assessed whether 2 different lipid emulsions can reverse cardiac electrophysiologic impairment induced by the IV administration of bupivacaine in anesthetized and mechanically ventilated piglets.

METHODS: Bupivacaine (4 mg · kg⁻¹) was injected over a 30-second period in 26 piglets. Thirty seconds after the end of bupivacaine injection, 1.5 mL · kg⁻¹ saline solution for the control group, and long-chain triglyceride emulsion (LCT group) or a mixture of long-chain and medium-chain triglyceride emulsion (LCT/MCT group) were infused over 1 minute. Cardiac conduction variables and hemodynamic variables were monitored for 30 minutes after injection.

RESULTS: Bupivacaine induced similar electrophysiologic and hemodynamic changes. After 3 minutes, His ventricle intervals (median and interquartiles) were 100 (85–105), 45 (35–55), and 53 (48–73) milliseconds in the control, LCT, and LCT/MCT groups, respectively (P < 0.001 between control and both lipid emulsion groups). Lipid emulsions also reversed the effects on QRS duration, atrial-His, and PQ (the onset of the P wave to the Q wave of the QRS complex) intervals. LCT/MCT emulsion restored the decrease in maximal first derivative of left ventricular pressure (P < 0.01 after 3 minutes versus control group).

CONCLUSIONS: LCT and LCT/MCT emulsions reversed the lengthening of His ventricle, QRS, atrial-His, and PQ intervals induced by the IV injection of 4 mg · kg⁻¹ bupivacaine. (Anesth Analg 2010;110:1473–9)

B upivacaine remains widely used for regional anesthesia because of its low cost and prolonged effectiveness.¹ However, cardiac arrests have been reported after accidental IV administration of bupivacaine.² A large dose of bupivacaine induces both a marked decrease in myocardial contractility and a dramatic slowing of ventricular conduction.³–⁸ This ventricular conduction impairment is induced by the decrease in maximal upstroke velocity of fast action potential in a dose- and use-dependent manner and can facilitate reentrant ventricular arrhythmias.⁹,¹⁰ Using a model of anesthetized and mechanically ventilated animals (dogs and piglets), we previously demonstrated that 4 mg · kg⁻¹ IV bupivacaine lengthens cardiac conduction and decreases myocardial contractility without inducing asystole, allowing reliable comparison of hemodynamic and electrophysiologic variables.¹¹–¹³

In animal studies, many drugs have been proposed to reverse the effects of bupivacaine-induced cardiotoxicity.¹¹,¹⁴,¹⁵ More recently, Weinberg et al.¹⁶–¹⁸ and Stehr et al.¹⁹ reported that rapid injection of long-chain triglycerides (LCTs; Intralipid®, Baxter Healthcare, Deerfield, IL) and a mixture of LCTs/medium-chain triglycerides (MCTs; Structitolipid®, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany), respectively, reversed cardiac arrest and myocardial depression induced by local anesthetics (bupivacaine in the studies by Weinberg et al. and levobupivacaine in the study by Stehr et al.). In humans, the successful use of LCT or LCT/MCT emulsions on cardiac arrhythmias has also been reported.²⁰–²³ Although LCT and LCT/MCT lipid emulsions have been shown to reverse local anesthetic hemodynamic impairment, only 1 study focused on the electrophysiologic variables altered by local anesthetics.¹⁹ In the study performed by Stehr et al.¹⁹ with low-dose levobupivacaine, no effect of LCT/MCT lipid emulsion on the QRS interval was reported.

Therefore, we used our model of anesthetized and mechanically ventilated piglets to test the effect of the 2 types of lipid emulsions previously studied (i.e., LCTs and mixture of LCTs/MCTs) on cardiac endocavitary electrophysiologic variables.¹²,¹³,¹⁶–¹⁹ The initial hypothesis was that both lipid emulsions could reverse the intracardiac electrophysiologic effects of 4 mg · kg⁻¹ bupivacaine.

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**METHODS**

The care and treatment of the animals used in this study complied with the national guidelines of the French Ministry of Agriculture (Paris, France). This study was approved by the regional ethics committee on animal studies (CE-LR-0805, April 11, 2008).

**Animal Preparation**

As previously described, 26 large white piglets (20–35 kg) were premedicated with an IM injection of ketamine (250 mg), midazolam (15 mg), and atropine (1 mg). Within 15 minutes, a peripheral catheter was inserted in an ear vein. The piglets were anesthetized with an IV sodium thiopental (10 mg·kg⁻¹ bolus followed by 15 mg·kg⁻¹·h⁻¹ until the end of the experiment). Tracheostomy was quickly performed, and the trachea was intubated. All piglets were then mechanically ventilated as follows: tidal volume = 10 mL·kg⁻¹, respiratory rate = 15 breaths·min⁻¹, and 21% oxygen (Siemens 900C respiratory ventilator, Siemens, Erlangen, Germany). Paralysis was maintained with IV cisatracurium (0.1–0.2 mg·kg⁻¹) as required. Body temperature was maintained at 38°C ± 0.5°C with a rewarming humidifier device. During the preparation and experiment (90–120 minutes), 500 to 750 mL of 0.9% sodium chloride was infused. In a previous study performed in the same animal model, it was demonstrated that cardiac electrophysiologic, hemodynamic, and biological variables were stable throughout a 2-hour period after the preparation period.12 Electrocardiographic recordings were taken from standard lead II. The right carotid artery was cannulated with a 6-French high-precision micromanometer (Millar Instruments, Houston, TX) that was advanced into the left ventricle to measure left ventricular pressure. A 6-French bipolar electrode catheter (USCI, C.R. Bard, Billerica, MA) was introduced via the femoral vein into the right ventricle to record the His bundle electrical activity.24 A 5-French catheter (PICCO, Pulsion Medical Systems AG, Munich, Germany) was inserted through the femoral artery into the descending aorta for arterial blood samples and cardiac output (CO) measurement by transpulmonary thermodilution. The right internal jugular vein was catheterized with a 7-French double-lumen catheter (Arrow, Erding, Germany) for measurement of central venous pressure (CVP) and infusion of medication. Once the catheters were in place, a 15-minute stabilizing period was allowed for all animals.

**Experimental Protocol**

Three groups of piglets were involved in this study. Piglets were randomized to receive isotonic saline solution for the control group, Lipid Emulsions and Bupivacaine Electrophysiologic Toxicity. Association of Anesthetists of Great Britain and Ireland (www.aagbi.org). No other treatment was administered during the experiment. After 30 minutes of experiment, all animals were euthanized with an overdose of sodium thiopental and potassium chloride. The laboratory personnel were not blinded to the treatment.

**Measured Variables**

Electrophysiologic data, intraventricular pressure, and CVP were recorded using the data acquisition system. Acknowledge MP 150 (BIOPAC Systems, Goleta, CA). The following electrophysiologic variables were measured (in milliseconds)\(^{11,12}\): cardiac cycle length (RR), PQ interval measured from the onset of the P wave to the Q wave of the QRS complex, atrial-His (AH) interval measured from the onset of atrial depolarization to the His bundle electrogram of the endocavitary lead, His-ventricle (HV) interval measured from the His bundle electrogram of the endocavitary lead to the Q wave of electrocardiogram lead II, QRS duration, QT interval, JT interval, and JT interval corrected by heart rate [JTc = (QT − QRS) × (RR)\(^{-0.5}\)]. The following hemodynamic variables were measured: mean aortic pressure (MAP, mm Hg), left ventricular end-diastolic pressure (LVEDP; mm Hg), maximal first derivative of left ventricular pressure (LdP/dt\(_{max}\); mm Hg·s\(^{-1}\)), CVP (mm Hg), and CO by transpulmonary thermodilution (L·min\(^{-1}\)). Blood samples were obtained to measure the following serum concentrations: sodium (Na\(^+\); mm), potassium (K\(^+\); mm), calcium (Ca\(^{2+}\); mm), protein (g·L\(^{-1}\)), hemoglobin (g·dL\(^{-1}\)), triglycerides (g·L\(^{-1}\)), and cholesterol (g·L\(^{-1}\)) (Architect C8000 Analyzer, Abbott Laboratories, Abbott Park, IL). Arterial blood gas analysis (arterial oxygen partial pressure in mm Hg, arterial carbon dioxide partial pressure in mm Hg, pH, arterial oxygen saturation, and CO\(_2\) total in mM) was performed using a RapidLab 405 automat (Siemens, Deerfield, IL). In all groups, the total plasma concentration of bupivacaine was measured using high-performance liquid chromatography and UV detection. The detection threshold was 0.5 µg·mL\(^{-1}\). The plasma samples were analyzed using the following high-performance liquid chromatography and UV detection methods.

**Times of Measurements**

In all groups, electrophysiologic and hemodynamic variables, except CO, were measured at baseline (T\(_0\)), 1 (T\(_1\)), 2 (T\(_2\)), 3 (T\(_3\)), 4 (T\(_4\)), 5 (T\(_5\)), 10 (T\(_{10}\)), 15 (T\(_{15}\)), and 30 (T\(_{30}\)) minutes after the beginning of the administration of bupivacaine. CO was measured at T\(_{1}r\), T\(_{15}r\), and T\(_{30}r\). Biological variables (Na\(^+\), K\(^+\), Ca\(^{2+}\), protein, hemoglobin, triglycerides, cholesterol, Pao\(_2\), Paco\(_2\), pH, arterial oxygen saturation, and CO\(_2\) total) were measured at T\(_{1}r\), T\(_{15}r\), and T\(_{30}r\). The total plasma concentration of bupivacaine was measured at T\(_{30}r\) by the end of the IV bolus of bupivacaine (T\(_{15}r\)), and at T\(_{15}r\), T\(_{15}r\), and T\(_{30}r\).

**Statistical Analysis**

Results are expressed as median with 25th and 75th percentiles (nonnormal distribution using Shapiro Wilks, kurtosis, and skewness tests). Because the 2 lipid emulsion compositions are similar and because of preliminary studies, our first hypothesis was that the difference between LCT and LCT/MCT emulsions was likely to be too small to be clinically relevant. Therefore, the primary goal of this study was to compare the electrophysiologic variables of
the control and both lipid emulsion groups without a comparison between lipid emulsions themselves. In previous studies, HV was the variable that was the most lengthened after bupivacaine injection from 25 to 105 milliseconds. As previously observed with ropivacaine (HV lengthening from 25 to 60 milliseconds) and a lidocaine-bupivacaine mixture (HV lengthening from 25 to 40 milliseconds), we assumed that a decrease of >50% of bupivacaine-induced HV lengthening between the control and both lipid emulsion groups was clinically relevant. Power analysis based on the results of previous studies yielded a sample size of 15 piglets for each group. A significance criterion was set at 0.05 and power at 0.8. However, because previous studies included 6 to 7 piglets with significant differences, an interim analysis was scheduled after the inclusion of 7 piglets per group, according to our regional ethics committee request. Even if we performed an interim analysis, we made the usual assumption that no adjustment of the statistical significance level was necessary.

Biological variables were compared between groups, using Wilcoxon-Mann-Whitney test, but we only considered clinically relevant differences because it was not the main objective of the study. Repeated measures, such as triglyceride concentrations, were compared between T0 versus T15 and T0 versus T30 using Wilcoxon paired test. The same test was used for bupivacaine-induced electrophysiologic and hemodynamic effects between T0 and T1. Moreover, bupivacaine plasma concentrations and hemodynamic and electrophysiologic variables between T1 and the end of the study were represented by the area under the curve (AUC, trapeze method). These AUC values were compared depending on the study group by using Wilcoxon-Mann-Whitney test, and the maximal or minimal concentration (Cmax or Cmin). When the overall evolution among the 3 groups was different, i.e., when the P value for AUC comparison was <0.05, a comparison was performed using a Friedman test for repeated measures from T1 to T30 to integrate both time and group effects. Furthermore, at each time, the Wilcoxon-Mann-Whitney test was performed to put the emphasis on the differences between groups. The significance level was set at 0.05. In case of subanalysis, it was corrected according to the Bonferroni correction. The analysis was performed using SAS v8.1 (SAS Institute, Cary, NC).

RESULTS

After the inclusion of 26 piglets, the interim analysis showed that both lipid emulsions could significantly reverse the cardiac electrophysiologic effects of bupivacaine compared with saline solution. As recommended by our regional ethic committee, these results led to the termination of the study. Two piglets in the LCT group could not be analyzed (1 piglet died during the preparation period and data were lost after a computer crash for the other one). Therefore, 9, 7, and 8 piglets were included in the control, LCT, and LCT/MCT groups, respectively.

Biological Variables

Biological variables were similar in all groups except for arterial oxygen saturation and pH, which were lower in the LCT/MCT group at T30 (P < 0.01 versus control group) (Table 1). Plasma concentrations of bupivacaine were similar in all groups and were >2 μg · mL⁻¹ throughout the experiment (AUC, P = 0.32; Cmax, P = 0.20) (Fig. 1). In both lipid emulsion groups, triglyceride plasma concentrations were 40 to 80 times higher than those in the control group (P < 0.01), with no significant difference between the LCT and LCT/MCT groups (Fig. 2).

Effects of Bupivacaine Injection Between T0 and T1

Bupivacaine induced a lengthening of HV (P < 0.01), QRS (P < 0.01), AH (P < 0.01), and PQ intervals (P < 0.01) in all groups, without significant alteration in RR and JTc intervals (Fig. 3).

Bupivacaine induced a decrease in LVdP/dtmax (P < 0.01) and an increase in LVEDP (P < 0.01) without a change in MAoP (Fig. 4) in all groups. No significant differences were found among the LCT, LCT/MCT, and control groups for electrophysiologic (HV, QRS, AH, PQ, RR, and JTc intervals) and hemodynamic (MAoP, LVEDP, LVdP/dtmax, CVP, and CO) variables between T0 and T1.

Effects of Lipid Emulsion Infusion

Lipid emulsions reversed the impairments of the HV, QRS, AH, and PQ intervals (Fig. 3). At T0, HV intervals were 100 (85–105), 45 (35–55), and 53 (48–73) milliseconds in the control, LCT, and LCT/MCT groups, respectively (P < 0.001, between the control and both lipid emulsion groups). No significant changes were shown in RR and JTc intervals.

LCT/MCT emulsion, but not LCT emulsion, reversed the decrease in LVdP/dtmax (Fig. 4). Indeed, although the AUC comparison for LCT emulsion (versus control) was statistically significant (P = 0.01), the Friedman test did not show a significant difference (P = 0.18). Concerning MAoP, despite differences in the AUC values (the AUC values of the LCT and LCT/MCT groups were significantly different from the AUC value of the control group), no significant difference was shown by the Friedman test (P = 0.19) (Fig. 4). CO, CVP, and LVEDP were not significantly different among groups.

DISCUSSION

This study shows that infusions of LCT and LCT/MCT lipid emulsions rapidly reverse the lengthening of atrioventricular and intraventricular conduction variables (PQ, AH, QRS, and HV) induced by the IV administration of 4 mg · kg⁻¹ bupivacaine.

Assessment of the Model

In this stable animal model of bupivacaine-induced cardiac electrophysiologic toxicity, an IV bolus dose of 4 mg · kg⁻¹ bupivacaine led to plasma concentrations >2 μg · mL⁻¹ in all piglets. This toxic dose of bupivacaine has been shown to induce slowing of atrioventricular and ventricular conduction impairment but no asystole that could alter the experimental model by introducing confounding factors. To mimic the clinical setting of an accidental IV administration of bupivacaine, lipid emulsions were infused at the rate recommended, 30 seconds after the end of bupivacaine injection. Finally, because no treatment other than lipid emulsions was given, we assume that our model allows a reliable comparison between control and treatments groups.
Effects of Lipid Emulsions on Electrophysiologic Variables

Few authors have studied the electrophysiologic effects of lipid emulsions in local anesthetic toxicity. In an isolated rat heart model, Stehr et al.\(^\text{19}\) showed that a low plasma levobupivacaine concentration (5 \(\mu\)g \cdot mL\(^{-1}\)) lengthens PQ and QRS intervals with no reversal effect of 1 LCT/MCT lipid emulsion (Structolipid), which has a composition similar to Medialipide. Indeed, our study is the first to...
show the efficiency of LCT and LCT/MCT lipid emulsions on endocavitary electrophysiologic variable impairments induced by a racemic bupivacaine plasma concentration.

Effects of Lipid Emulsions on Hemodynamic Variables

Weinberg et al.16–18 initially reported the beneficial effects of LCT lipid emulsion on bupivacaine-induced hemodynamic impairment in different experimental models. The present study confirms these findings because MAoP and LVDp/dt max were increased by the infusion of lipid emulsions when the comparison with AUC was performed. Using the Friedman test, the only significant hemodynamic improvement was LVDp/dt max in the LCT/MCT group. On one hand, this finding could be partly explained by a lack of statistical power because the experiment was conducted to explore endocavitary electrophysiologic alterations. On the other hand, lipid nanoemulsions (diameter of particle <118 nm) have been shown to better reverse bupivacaine-induced QRS-interval prolongation in an isolated heart model.30 Because LCT and LCT/MCT lipid emulsions have different particle sizes (i.e., 430 nm vs 280 nm, respectively), they could have different effects on bupivacaine-induced cardiotoxicity. However, free bupivacaine plasma concentrations were not measured and therefore this study could not confirm this hypothesis. Furthermore, the statistical power was
not sufficient to evaluate differences between lipid emulsions.

**Study Limitations**

There are several limitations to our study. First, our power analysis was based on a clinically relevant difference in electrophysiologic variables expected between saline and lipid emulsions, not between the 2 lipid emulsions. Second, the effect of premedication or anesthesia can be questioned. However, the differences observed among groups were only caused by the direct cardiac effect of bupivacaine and lipid emulsions. Third, the effect of heart rate on bupivacaine intoxication (use dependence) could not be studied because no atrial pacing was achieved. However, no difference in heart rate was observed among groups. Finally, we did not assess the mechanisms that ensure the beneficial effects of lipid infusion. Mazoit et al. recently reported that LCT emulsions were more efficient than LCT/MCT emulsion to bind long-acting local anesthetics. Because of conflicting results, extrapolations from this study to humans should be made with caution.

**Clinical Implications**

This study shows that LCT and LCT/MCT lipid emulsions reverse the prolongation of cardiac conduction intervals caused by bupivacaine intoxication. This finding strengthens the recommendations published by the Association of Anesthetists of Great Britain and Ireland. Therefore, when an accidental IV injection of local anesthetics with cardiac conduction lengthening is diagnosed, the infusion when an accidental IV injection of local anesthetics with cardiac conduction lengthening is diagnosed, the infusion of 1.5 mL·kg⁻¹ (nearly 100 mL for a human weighing 60–70 kg) of 1 lipid emulsion over a 1-minute period could reverse cardiac toxicity. However, as found in our study, the use of lipid emulsions in the case of central nervous system toxicity without cardiac symptoms must be balanced with the potential effect on gas exchange. More importantly, the efficiency of lipid emulsion does not obviate the need for cardiopulmonary resuscitation, immediate oxygenation, and tracheal intubation in case of cardiac arrest.

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