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Bupivacaine Toxicity Increases Transmural Dispersion of Repolarization, Developing of a Brugada-like Pattern and Ventricular Arrhythmias, Which is Reversed by Lipid Emulsion Administration. Study in an Experimental Porcine Model

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Abstract

Unintentional administration of bupivacaine may be associated with electrocardiogram changes that promote the development cardiac arrhythmias. Ventricular repolarization markers (corrected QT, QT dispersion, Tpeak–Tend and Tpeak–Tend dispersion) are useful to predict cardiac arrhythmias. We aim to investigate the effects of bupivacaine on the transmural dispersion of repolarization and their reversion following intravenous lipid emulsion (ILE) administration. Fourteen pigs were anaesthetized with thiopental and sevoflurane and underwent tracheal intubation. After instrumentation, a 4 mg kg-bolus of bupivacaine was administrated followed by an infusion of 100 μg kg^{-1} min^{-1}. QT interval, QTc:QT corrected by heart rate, Tpeak-to-Tend interval and QT and Tpeak-to-Tend dispersion were determined in a sequential fashion: after bupivacaine (at 1 min, 5 min and 10 min) and after ILE (1.5 mL kg^{-1} over 1 min followed by an infusion of 0.25 mL kg^{-1} min^{-1}). Three additional animals received only ILE (control group). Bupivacaine significantly prolonged QT interval (Δ:36%), QT dispersion (Δ:68%), Tpeak-to-Tend (Δ:163%) and Tpeak-to-Tend dispersion (Δ:98%), from baseline to 10 min. Dispersion of repolarization was related to lethal arrhythmias [three events, including asystole, sustained ventricular tachycardia (VT)] and repeated non-sustained VT (4/14, 28%). A Brugada-like-ECG pattern was visualized at V1–4 leads in 5/14 pigs (35%). ILE significantly decreased the alterations induced by bupivacaine, with the termination of VT within 10 min. No ECG changes were observed in control group. Bupivacaine toxicity is associated with an increase of transmural dispersion of repolarization, the occurrence of a Brugada-like pattern and malignant VA. ILE reverses the changes in dispersion of repolarization, favouring the disappearance of the Brugada-like pattern and VT.

Keywords Bupivacaine · Transmural dispersion of repolarisation · Brugada-like · Lipid emulsions

Introduction

Unintentional intravenous administration of bupivacaine might produce life-threatening ventricular arrhythmias and subsequent cardio-depression [1, 11, 12]. Myocardial

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sodium channel blockage is the main mechanism of bupivacaine cardiac toxicity, leading to an increase in PR and QRS intervals [10, 26, 28]. Moreover, cardiac potassium and calcium ion channels could also be affected by toxic doses of bupivacaine [22–24, 26]. As a result of these complex ion channel interactions, bupivacaine in toxic concentrations modifies the action potential duration, which is reflected as a QT interval lengthening in an electrocardiogram (ECG) [7].

Typically, repolarization of the ventricular myocardium occurs asynchronously and is reflected in the morphology of the T wave of the ECG. When the epicardium has been completely repolarized, the peak of the T wave is inscribed. However, the end of the T wave coincides with the repolarization of the midmyocardial region that repolarizes later. Consequently, the interval between the peak and the end of the T wave (Tpeak-to-Tend) is a useful index that reflects the transmural dispersion of repolarization (TDR) and its prolongation contributes to creating an arrhythmogenic substrate that can lead to ventricular arrhythmias, such as "torsades de pointe" (TdP) [2–4, 9, 25–27, 30].

The effect of bupivacaine on the QT interval has been previously reported. However, no studies have investigated the effects of bupivacaine on the parameters of TDR, which are more relevant to evaluate the risk of arrhythmias induced by bupivacaine [5, 8, 18].

Experimental and clinical case reports have shown that intravenous lipid emulsion (ILE) can be effective in reversing bupivacaine cardiovascular toxicity [7, 12–14, 20, 31]. Nevertheless, the effect of ILE on TDR has not been studied.

The main aim of the current study was to investigate the effects of bupivacaine on parameters related to TDR. As a secondary aim we wanted to evaluate the effects of ILE on the TDR effects induced by bupivacaine.

Methods

The Animal Ethic Committee of the medical laboratory of experimental medicine approved the study, (February-20-2013; ACTA CEEA nº 1. 2013). The experimental procedures employed in this study were carried out according to de Guide for the Care and use of Laboratory Animals. All applicable international, national, and/or international care and use of animals were followed.

The study was performed using large-white-pigs. After overnight fasting, (we allowed free access to water until 2 h before anaesthesia) the pigs were premedicated using 20 mg kg^{-1} of ketamine intramuscularly [6]. The animals were provided with 100% oxygen via a facial mask, and anaesthesia was induced by injection of sodium thiopental at 5–10 mg kg^{-1}. The trachea was intubated and the lungs were ventilated using a volume-controlled ventilator (Dräger SA1 ventilator, Dräger Medical Hispania, S.A. Spain) with pure oxygen and at a ventilatory frequency sufficient to maintain normocapnia. During the procedure, the animals received an infusion of 0.9% saline solution at 5 mL kg^{-1} h^{-1}. The anaesthesia was maintained using sevoflurane 1 MAC, as referred to in the appropriate literature (1 MAC = 2.66%) [15]. Pulse oximetry, end-tidal sevoflurane, CO_2 and O_2 were continuously monitored. The temperature was maintained close to 38 °C, using a forced-air warmed blanket (Warm Touch, Patient Warming System; Mallinckrodt Medical) when necessary.

The femoral vessels were catheterized and arterial pressure was registered by means of an intraarterial catheter and continuously recorded using a polygraph monitor (LABSYSTEM PRO™ EP Recording System, Boston Scientific Corporation, Massachusetts, US). Blood arterial samples were obtained at baseline and at intervals throughout the experiment for gas analysis and for bupivacaine plasma determination. A 12-lead ECG simultaneous recording was performed and the data were continuously recorded using a polygraph monitor. The electrodes were positioned as follows: V1 at the lower right sternum, V2 at the lower left sternum and the subsequent V3–V6 electrodes were positioned equidistantly across the lateral left hemithorax. After instrumentation, a 10 min stabilizing period was allowed before initiating the experimental procedure.

Experimental Protocol

A modification of a previous porcine experimental bupivacaine intoxication model was performed [31]. Our intention was to create a stable model of bupivacaine toxicity that induces electrical cardiac toxicity, without causing asystole or severe myocardial depression. A bolus of 4 mg kg^{-1} of bupivacaine (Bupivacaine HCl 0.75%, Fresenius Kabi, Spain) was administered followed by a continuous infusion of 100 µg kg^{-1} min^{-1}, via the peripheral ear vein. In five animals, 10 min after the bupivacaine infusion, ILE (20% Intralipid®; Fresenius Kavi AB, Uppsala, Sweden) was infused at 1.5 mL kg^{-1} over 1 min, followed by an infusion of 0.25 mL kg^{-1} min^{-1}. The dose of Intralipid was based on the guidelines published by anaesthetic societies [14].

A control group of three animals in which bupivacaine was replaced by an identical volume of normal saline was included. In addition, 3 animals received only Intralipid to explore the possible impact of ILE on the electrocardiographic parameters.

A schematic flowchart of the study protocol is shown in Fig. 1. At the end of the experiment, the animals were euthanized using 10 mg/kg of propofol and potassium chloride.

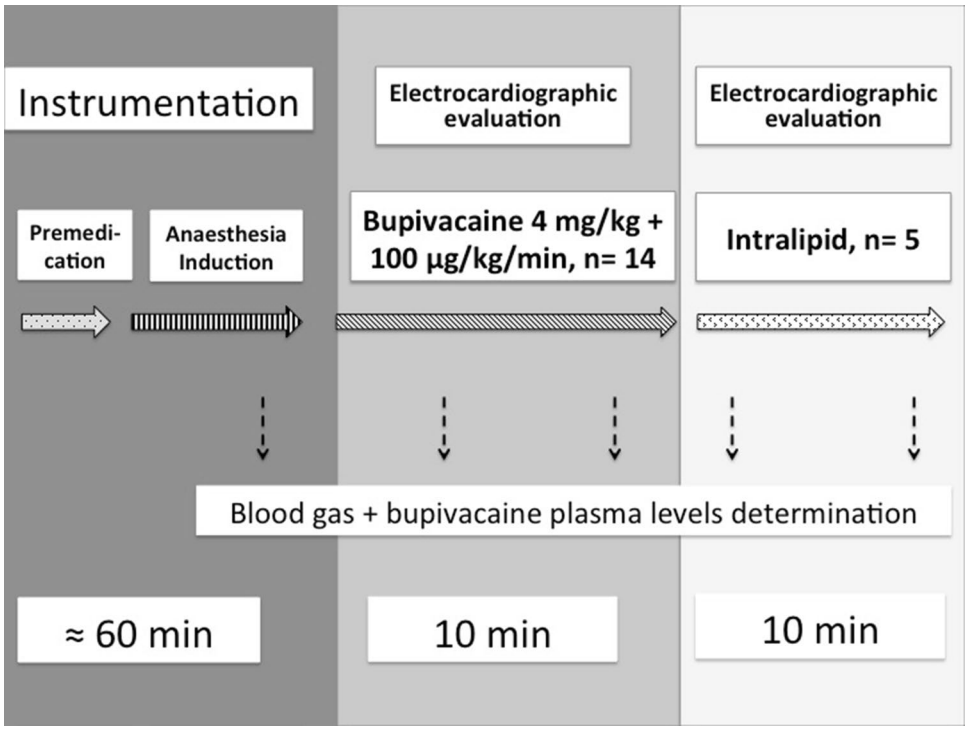

Fig. 1 Study design with the mean duration time of the different phases of the protocol. Electrocardiographic evaluations were performed during bupivacaine administration and after Intralipid. Arrows represent blood gas sampling times and plasma bupivacaine determination

Electrocardiographic Measurements

The following electrocardiographic measurements were performed:

- *QT interval* from the onset of the QRS complex to the end of the T wave (defined as the junction of the isoelectric line and the tangent of the maximal downward limb of the T wave).
- *Corrected QT interval* calculated according to the Bazett's formula: QT interval divided by the square of the RR intervals.
- *Tpeak-to-Tend interval* from the peak of the T wave to the end of the T wave.

Parameters of TDR

- *QT dispersion* The difference between maximum and minimum QT intervals in the 12-lead ECG.
- *Tpeak-to-Tend dispersion* The difference between maximum and minimum Tp-e intervals in the 12-lead ECG.

Ventricular Arrhythmias

Non-sustained ventricular tachycardia was defined as a run of at least four uniform, repetitive extrasystoles with an RR > 100 beats/min and lasting < 30 s.

Sustained ventricular tachycardia was defined as a run of repetitive extrasystoles with an RR > 100 beats/min and lasting > 30 s.

All measurements were performed manually, magnifying the size of the ECG recording to avoid errors. The QT and the Tpeak-to-Tend intervals were measured in each lead and averaged to give a mean QT and Tpeak-to-Tend interval. Two investigators (MZ and DD C) separately analysed the ECG traces according to the previously defined criteria.

Electrocardiographic parameters were recorded before administration of bupivacaine, and 5 and 10 min after bupivacaine injection. In the animals that received ILE, the parameters were recorded 10 min after the lipid administration.

The onset of arrhythmias was also noted for each animal.

Bupivacaine Determination

The total plasma bupivacaine concentration was determined using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) as previously described [31].

Statistical Analysis

The normal distribution of variables was assessed using the Kolmogorov–Smirnov test. Continuous data are expressed as the mean ± standard deviation (SD) or median and the corresponding ranges. The effect of bupivacaine on electrocardiographic intervals and hemodynamic parameters was analysed using a one-way repeated-measures ANOVA. Electrocardiographic parameters in intralipid and saline control groups were compared with baseline values using paired Wilcoxon test. Blood gas analysis was performed using paired Student's *t*-test.

Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using the SPSS-20 software package.

Sample Size

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 12 animals were necessary to recognize as statistically significant a difference greater than or equal to 30 ms in Tpeak-to-Tend interval after bupivacaine administration. The standard deviation was 35 ms. A drop-out rate of 10% was anticipated.

Results

A total of 20 pigs, with a mean weight of 42 ± 11 kg, were studied. No animals died because of instrumentation. However, two animals presented severe hemodynamic alterations after bupivacaine infusion.

Hemodynamic data, blood gas analysis and electrolytes are shown in Table 1. No significant changes were found in mean systolic and diastolic blood pressure throughout the study period. A significant reduction in heart rate was seen at 5 and 10 min following bupivacaine infusion. Biological variables were not significantly altered throughout the study period, except a clinically irrelevant increase in sodium concentration 10 min after bupivacaine administration.

Plasma bupivacaine levels are shown in Fig. 2. The mean bupivacaine levels remained in the toxic range from the end of the bupivacaine infusion to 20 min later. At 30 min, the mean plasma bupivacaine level was 1.5 ± 0.6 µg mL^{-1}, a concentration associated with less electrocardiographic and hemodynamic effects.

The electrocardiographic changes induced by bupivacaine are shown in Table 2. The administration of bupivacaine prolonged all parameters of TDR. The Tpeak-to-Tend interval showed the greatest increase of 163% from baseline ($p = 0.001$) followed by the Tpeak-to-Tend dispersion that increased by 98%. Figure 3 shows an example of the Tpeak-to-Tend interval lengthening after bupivacaine administration. Figure 4 shows the evolution of the T peak-to-Tend interval and the QTc interval throughout the experiment.

Table 1 Haemodynamic data, arterial blood gases and electrolytes

	Baseline	5 min bupivacaine	10 min bupivacaine	*p*
SBP (mmHg)	107 ± 21	90 ± 26	95 ± 24	0.26
DBP (mmHg)	65 ± 14	51 ± 18	55 ± 19	0.22
HR (bpm)	100 ± 19	82 ± 20	83 ± 18	0,04
pH	7.51 ± 0.05	–	7.48 ± 0.04	0.11
PaO_2 (mmHg)	464 ± 126	–	495 ± 62	0,77
$PaCO_2$ (mmHg)	37 ± 4.1	–	37 ± 2.7	0.89
HCO_3^- (mmol L^{-1})	30 ± 2.1	–	30 ± 1.7	0.47
BE (mmol L^{-1})	8.9 ± 3.4	–	6.5 ± 2.3	0.08
SaO_2 (%)	100	–	100	–
Na^+ (mmol L^{-1})	139 ± 1.9	–	140 ± 2	0.01
K^+ (mmol L^{-1})	3.4 ± 0.2	–	3.5 ± 0.4	0.20
Ca^{++} (mmol L^{-1})	1.2 ± 0.07	–	1.2 ± 0.06	0.3
Haematocrit (%)	27 ± 2.4	–	25 ± 2.4	0.13

Values are mean ± SD

DBP diastolic blood pressure; *HR* heart rate; *SBP* systolic blood pressure

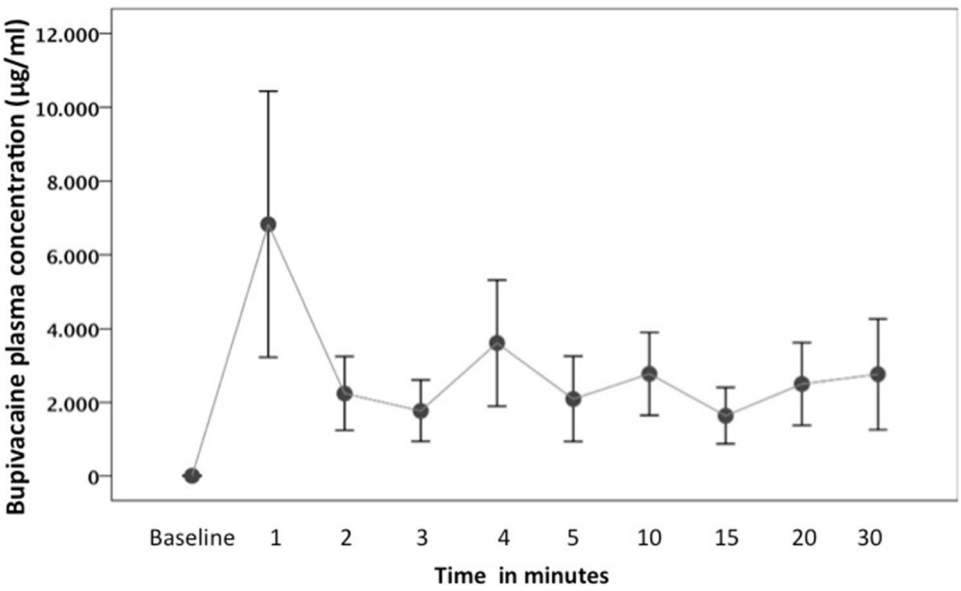

Fig. 2 Bupivacaine plasma levels during the study

Table 2 Electrocardiographic data after bupivacaine administration

	Baseline	1 min BUPI	5 min BUPI	10 min BUPI	Mean % of difference[a]	*p*
QT interval (ms)	382 ± 39	448 ± 42	481 ± 41	519 ± 65	36	0.0001
QT dispersion	50 ± 20	79 ± 30	83 ± 26	84 ± 40	68	0.04
QTc (ms)	501 ± 29	519 ± 45	554 ± 73	597 ± 78	19	0.01
Tpeak-to-Tend interval (ms)	60 ± 12	132 ± 33	153 ± 30	158 ± 31	163	0.0001
Tpeak-to-Tend dispersion	43 ± 20	65 ± 21	76 ± 40	85 ± 33	98	0.02

Values are mean ± SD

Repeated measures of ANOVA

[a]From baseline to 10 min after bupivacaine administration

Arrhythmias Induced

The administration of bupivacaine was related to arrhythmias in six animals, two of them presented lethal events: asystole and sustained ventricular tachycardia and four repeated of them presented non-sustained ventricular arrhythmias (28%).

A Brugada-like ECG pattern was visualized at leads V1–4 in 5/14 pigs (36%). Figure 5 shows an example of this electrocardiographic phenomenon.

Intralipid decreased the lengthening of the TDR parameters induced by the administration of bupivacaine (Table 3). Moreover, intralipid restored sinus rhythm within 10 min in two of the animals that developed arrhythmias. Intralipid administration and resuscitation manoeuvres allowed for recovery from cardiac arrest in two animals.

In Tables 4 and 5 are shown the electrocardiographic parameters that result in saline and ILE control groups. No differences in electrocardiographic parameters were observed. No arrhythmias were presented by any of the pigs.

Discussion

The main finding of the current study is that bupivacaine 4 mg kg^{-1} induces an intense effect on the parameters of TDR, including a Brugada-like ECG pattern. Moreover, bupivacaine facilitated the occurrence of ventricular arrhythmias in a significant proportion of animals (43%). Finally, our study shows further evidence of the efficacy of ILE in reverting the effect of bupivacaine on repolarization, and in the control of induced arrhythmias.

Conflicting effects of bupivacaine on the QT interval have been described. Some studies have reported a shortening of the corrected QT interval, whereas other authors have observed an increase in the QT interval [16–18]. Studies that have evaluated the effect of bupivacaine on repolarization based on QT interval measurements have some limitations. As the QT interval reflects both ventricular repolarization and depolarization, and a widening of the QRS is observed with the administration

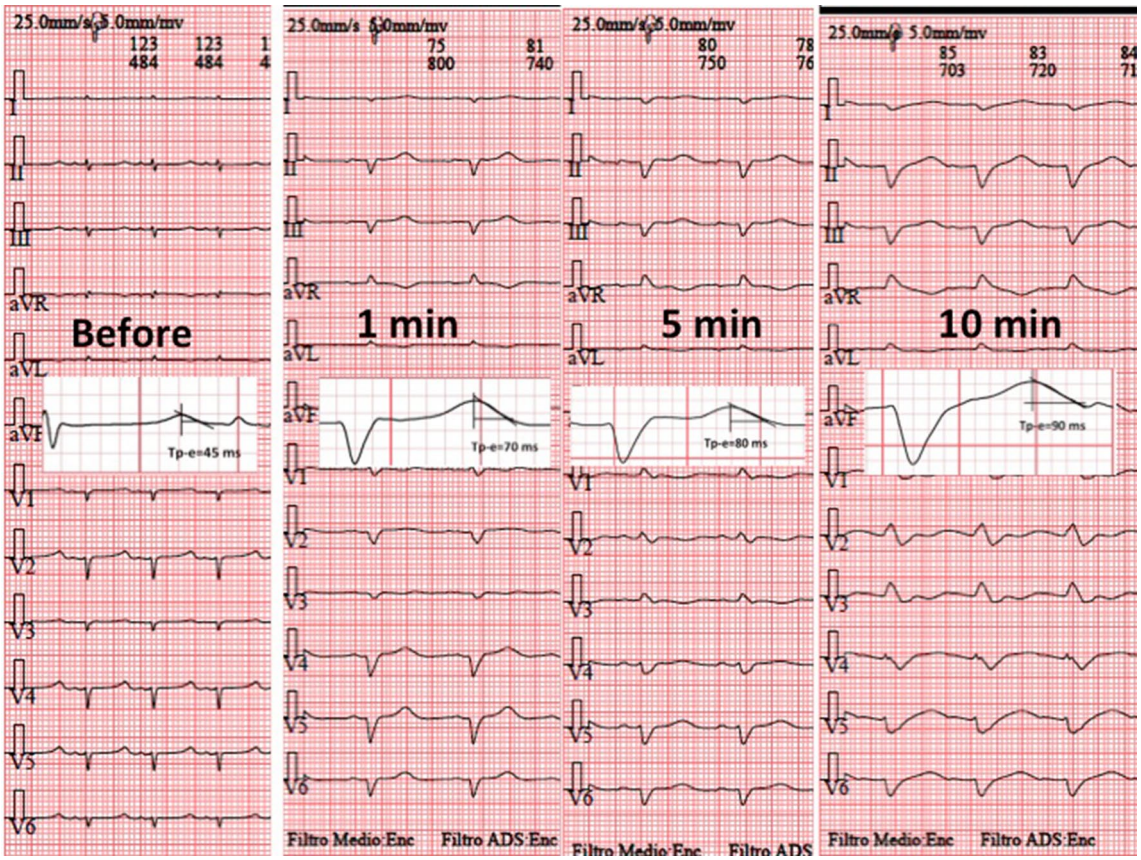

Fig. 3 The effect of bupivacaine 4 mg kg^{-1} on Tp–e interval. A huge increase on the Tp-e interval was observed from 40 ms at baseline to 150 ms 10 min after bupivacaine administration

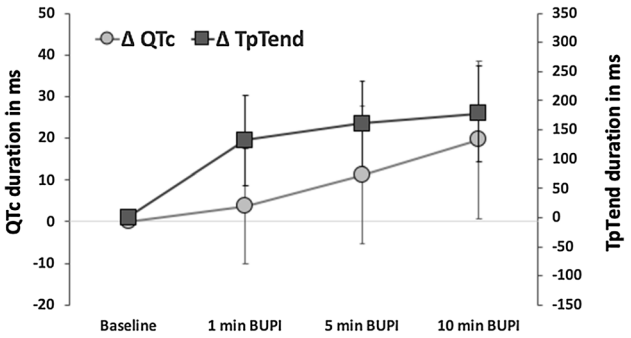

Fig. 4 Evolution of the TpTend and QTc intervals after the administration of bupivacaine. Note that the progressive increase in the TpTend interval is followed by a concomitant increase in QTc interval

of bupivacaine, an increase in the QT interval could be caused by the concomitant widening of the QRS. In contrast, the T wave represents ventricular repolarization, and the Tpeak–Tend interval corresponds to the dispersion of repolarization. The ventricular myocardium is composed of at least three distinct cell types: epicardium, M cells (deep subendocardium) and endocardium, which differ from each other in their repolarization behaviour. The M cells demonstrate a higher vulnerability to a prolonged action potential in response to agents that prolong the action potential, compared with epicardial and endocardial cells. The end of the repolarization of epicardial cells coincides with the peak of the T wave (T_{peak}), the end of the repolarization of endocardial cells coincides with the end of the T wave (T_{end}) and the repolarization of M cells is aligned with the end of the T wave [2]. TDR plays an important role in arrhythmogenesis. Recent studies have shown that an increase in the Tpeak–Tend interval is associated with a high risk for the development of TdP in patients with long QT syndrome (LQTS) [2, 4]. This phenomenon could favour the development of ventricular arrhythmias through two mechanisms; first, the descending limb of the T wave represents a vulnerable period in which any electrical impulse could trigger malignant arrhythmias (i.e. LQTS, myocardial ischemia, Brugada syndrome), and secondly, the increase in the TDR may result in conduction block in the area with longer repolarization and generate a functional re-entrant substrate and the consequent development of malignant arrhythmias. In the current study, the maximum increase in the

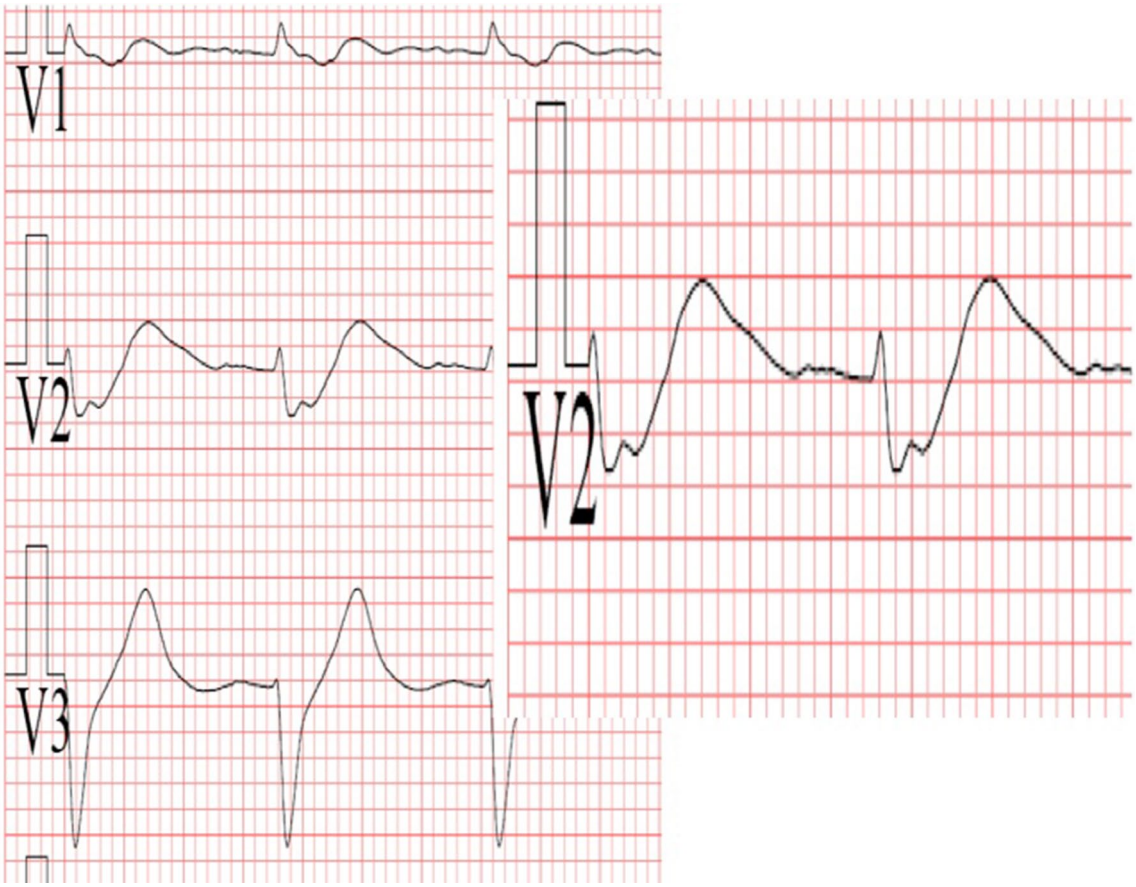

Fig. 5 Brugada-like ECG pattern observed 1 min after the infusion of a bupivacaine bolus of 4 mg kg^{-1}

Table 3 Electrocardiographic data after intralipid administration ($n=5$)

	End of infusion of bupivacaine	10 min intralipid	p^a
QT interval (ms)	481 ± 24	459 ± 102	0.5
QT dispersion	82 ± 24	34 ± 25	0.08
QTc (ms)	585 ± 61	522 ± 97	0.13
Tpeak-to-Tend interval (ms)	147 ± 26	76 ± 8	0.04
Tpeak-to-Tend dispersion	83 ± 26	38 ± 20	0.08

Values are mean ± SD

[a]Compared with the end of bupivacaine infusion

Tpeak–Tend interval was observed from 10 min following the bupivacaine infusion and the mean value was 158 ms. In humans, a Tpeak–Tend interval of 117 ms is considered the best discriminator of the risk of TdP in susceptible patients, and a prolonged Tpeak–Tend interval is associated with the spontaneous development of arrhythmias in patients with organic heart disease [25–27]. In the current study, 43% of the animals developed arrhythmias. An alteration in conduction, caused by the blockade

Table 4 Electrocardiographic in control group only intralipid administration ($n=3$)

	Baseline	10 min intralipid	p^a
QT interval (ms)	415 ± 85	425 ± 96	0.28
QT dispersion	39 ± 13	29 ± 4	0.28
QTc (ms)	497 ± 34	490 ± 43	0.2
Tpeak-to-Tend interval (ms)	55 ± 8	52 ± 6	0.1
Tpeak-to-Tend dispersion	23 ± 3	19 ± 10	0.28

[a]Compared with baseline

Table 5 Electrocardiographic data in control group (saline) n = 3

	Baseline	10 min BUPI	p^a
QT interval (ms)	361 ± 47	377 ± 45	0.10
QT dispersion	32 ± 8	28 ± 6	0.65
QTc (ms)	439 ± 57	474 ± 36	0,10
Tpeak-to-Tend interval (ms)	44 ± 6	52 ± 8	0.10
Tpeak-to-Tend dispersion	15 ± 8	19 ± 10	0.65

Values are mean ± SD

[a]Compared with baseline

of cardiac sodium channels, is considered the principal arrhythmogenic mechanism of bupivacaine [10]; however, the intense effect on repolarization seen in the current study could have played a synergistic role in the arrhythmias observed. To the best of our knowledge, this is the first study to evaluate the effect of ILE on TDR induced by bupivacaine, and our data support the use of ILE to combat these cardiotoxic alterations associated with bupivacaine intoxication. Currently, a multimodal mechanism underlying lipid reversal of local anaesthetic toxicity is proposed: the lipid sink theory, or "shuttle" effect, that states that ILE enhances the removal of bupivacaine (highly lipid-soluble local anaesthetic) from cardiac tissues into the lipid emulsion of the plasma, being then transferred to other tissues such as adipose and muscle. Also, lipid administration would improve cardiac output and blood pressure through actions on the heart, in addition to the inhibition of severe vasodilation induced by bupivacaine. Finally, a postconditioning benefit via activation of prosurvival kinases is a plausible but somewhat hypothesis [12, 13, 20].

An interesting finding in the current study is the Brugada-like ECG pattern observed in 36% of the animals. A possible explanation is that as bupivacaine is a potent sodium channel blocker, the toxic doses administered could unmask a Brugada-ECG pattern in susceptible animals. The Brugada syndrome is characterized by a significant increase in TDR and it has been demonstrated that an increase in Tpeak–Tend and Tpeak–Tend dispersion is associated with a higher incidence of recurrent arrhythmias [19, 26, 27]. Two clinical reports have described the development of a Brugada-like ECG pattern after an epidural infusion of bupivacaine [21]. Moreover, in one of these reports, the patient presented with a ventricular tachycardia and electrical storm following the change in his basal ECG to a Brugada-like ECG pattern. The arrhythmia was successfully treated using lidocaine and amiodarone. Subsequently, the authors confirmed that a genetic mutation of the sodium channel was present in this patient. To evaluate the effects of bupivacaine on electrophysiologic parameters related with the Brugada syndrome, these authors conducted an electrophysiologic study in five ventricular wedge preparations from the right ventricle of dog hearts. They demonstrated that bupivacaine created a transmural and epicardial dispersion of repolarization that justified the Brugada-ECG changes and arrhythmias observed in this particular patient [29]. This phenomenon, observed in a higher proportion of the pigs in the current study, suggests that cautious use of bupivacaine in susceptible patients is warranted. An interesting finding is the favourable evolution of the Brugada pattern and the reversal of arrhythmias with the administration of Intralipid.

Conclusions

Bupivacaine toxicity in this porcine model was associated with an increase of TDR, the occurrence of a Brugada-like pattern and malignant ventricular arrhythmias. Intravenous lipid emulsion reversed the changes in the dispersion of repolarization, favouring the disappearance of the Brugada-like pattern and ventricular arrhythmias.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest related with this manuscript.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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