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ORIGINAL ARTICLE

Effect of lipid emulsion on the central nervous system and cardiac toxicity of bupivacaine and levobupivacaine in awake rats

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Abstract
Purpose Despite numerous studies examining the effect of lipid emulsion on bupivacaine-induced cardiac toxicity, few studies have examined its effect on central nervous system (CNS) toxicity of local anesthetics. We investigated the effect of lipid emulsion on the CNS and cardiac toxicity of bupivacaine and levobupivacaine in awake, spontaneously breathing rats.
Methods Male Sprague–Dawley rats were randomly allocated to control–bupivacaine (CB), control–levobupivacaine (CL), lipid–bupivacaine (LB), and lipid–levobupivacaine (LL) groups ($n = 8$ in each group). After infusion of saline (CB and CL groups) or 20 % lipid emulsion (LB and LL groups) for 5 min, bupivacaine (CB and LB groups) or levobupivacaine (CL and LL groups) was administered IV at 1 mg/kg/min. Cumulative dose of anesthetics and their plasma concentrations at the onset of convulsions and cardiac arrest were measured.
Results The doses of bupivacaine for inducing convulsions and cardiac arrest in the LB group (8.8 ± 1.7 and 10.2 ± 1.5 mg/kg, respectively) were significantly larger than those in the CB group (5.9 ± 1.1 and 7.1 ± 1.3 mg/kg, respectively, $p < 0.001$ for both). The doses of levobupivacaine for inducing convulsions and cardiac arrest in the LL group (10.0 ± 2.0 and 13.7 ± 3.6 mg/kg, respectively) were significantly larger than those in the CL group (7.7 ± 1.6 and 9.4 ± 2.4 mg/kg, $p = 0.03$ and $p = 0.02$, respectively). Plasma concentrations of bupivacaine at the onset of convulsions and cardiac arrest in the LB group (12.9 ± 2.9 and 41.4 ± 5.2 μg/ml, respectively) were significantly higher than those in the CB group (7.9 ± 1.2 and 21.6 ± 3.3 μg/ml, respectively, $p < 0.001$ for both). Plasma concentrations of levobupivacaine at the onset of convulsions and cardiac arrest in the LL group (17.5 ± 1.5 and 47.6 ± 6.1 μg/ml, respectively) were significantly higher than in the CL group (10.9 ± 2.2 and 29.2 ± 3.5 μg/ml, respectively, $p < 0.001$ for both).
Conclusions Lipid emulsion decreased CNS and cardiac toxicity of both bupivacaine and levobupivacaine.

Keywords Bupivacaine · Levobupivacaine · Central nervous system toxicity · Cardiac toxicity · Lipid emulsion

Introduction

The effect of lipid emulsion on local-anesthetic-induced cardiac toxicity has been extensively studied [1]. Most of those studies employed anesthetized, mechanically ventilated animal models [2–4]. One reason for this is to avoid the effect of central nervous system (CNS) toxicity on the heart. Large-dose local anesthetics are usually used for regional anesthesia in awake patients in the clinic, CNS toxicity is induced by smaller doses of local anesthetics than is cardiac toxicity, and symptoms such as dizziness, excitation, loss of consciousness, and convulsions often precede dysrhythmia, hypotension, and cardiac arrest [5–9].

As lipid emulsion effectively decreases cardiac toxicity induced by local anesthetics, it would also be effective for

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reducing CNS toxicity as described in those case reports; however, no studies have examined the effect of lipid emulsion on CNS toxicity of local anesthetics. Also, most experiments examining the effect of lipid emulsion on local anesthetic-induced toxicity have been performed on racemic bupivacaine [2, 3], and there is a paucity of data regarding levobupivacaine—a stereoisomer of bupivacaine with less toxicity [10, 11]—despite successful treatment in a patient of levobupivacaine-induced CNS and cardiac toxicity by lipid emulsion [7].

In this study, we sustained the hypothesis that lipid emulsion might increase the threshold for bupivacaine-induced CNS and cardiac toxicity and also examined the effect of lipid emulsion on levobupivacaine toxicity.

Methods

Animal preparation

After approval from the Institutional Animal Care and Use Committee, 32 male Sprague–Dawley rats aged 10 weeks and weighing 350–380 g (Clea Japan, Inc., Tokyo, Japan) were included in this study. Rats were anesthetized 2 or 3 days before the experiment with intraperitoneal ketamine, and bipolar electrodes for measuring electroencephalogram (EEG) (Unique Medical Co., Ltd., Tokyo, Japan) were installed so that the tips were placed at the nucleus accumbens using a stereotaxic instrument (Narishige, Tokyo, Japan) following the method reported previously [12]. Electrode positions were checked by thin slices of the brain removed after experiments. On the day of experiments and under general anesthesia with sevoflurane, the carotid artery and cervical and femoral veins were cannulated with polyethylene catheters for monitoring mean arterial blood pressure (MAP) and heart rate (HR) and for blood sampling and drug infusion, as reported previously [13]. These catheters were tunneled subcutaneously to the posterior cervical region so that the animals could move freely. Before emergence from anesthesia, the animals were placed in a plastic container to recover for at least 4 h before the experiment. During that time, the arterial catheter was connected to a pressure transducer, and MAP and HR were recorded continuously on a polygraph (RM-6000; Nihon Kohden, Tokyo, Japan) connected to a computer. HR was monitored with cardiotachometer triggered by arterial pressure.

Experimental procedure

The animals were divided into four groups ($n = 8$ in each group). After baseline measurement and blood sampling, each rat was randomly assigned to receive either saline [control–bupivacaine (CB) and control–levobupivacaine (CL) groups] or 20 % lipid emulsion (Intralipid, Frezenius Kabi, Tokyo, Japan) [lipid–bupivacaine (LB) and lipid–levobupivacaine (LL) groups] at 3 ml/kg/min for 5 min. Infusion rate and duration of lipid emulsion was determined based on the experiments reported by Weinberg [2]. Following infusion of saline or lipid emulsion, infusion of bupivacaine (CB and LB groups) or levobupivacaine (CL and LL groups) was started at 1 mg/kg/min. Infusion rate of local anesthetics was determined based on our previous studies suitable to observe the onset of CNS toxicity [13] and was continued until blood pressure (BP) waves disappeared. BP, HR, and EEG were continuously monitored, and the onset of convulsions was detected by the appearance of tonic–clonic movement of the legs and spike waves on EEG. Observation of rats was performed by one of the authors (YO), who was unaware of group allocation.

Arterial blood (0.3 ml) was drawn before infusion of saline or lipid emulsion (baseline), before starting infusion of bupivacaine or levobupivacaine, and at the onset of convulsions for blood gas analysis. At the onset of convulsions and cardiac arrest, 0.5 ml blood was obtained to measure plasma concentrations of local anesthetics. Blood gas was measured immediately after sampling with a blood gas analyzer (ABL4; Radiometer, Copenhagen, Denmark). Remaining blood samples were centrifuged at 12,000 g to eliminate lipid emulsion, and plasma was frozen and kept at −80 °C until analysis.

Measurement of local anesthetics

Plasma concentrations of bupivacaine and levobupivacaine were measured by 4,000 Qtrap tandem mass spectrometry (Applied Biosystems, Foster City, CA, USA), as reported previously [14]. In our preliminary experiments, infusion of lipid emulsion did not affect the measurement of plasma concentration of bupivacaine within the range of 0.1–50 μg/ml. The lower limit of quantitation was 1 ng/ml. Coefficients of within-day and between-day variation were 4.8 % and 3.8 %, respectively.

Statistics

The number of animals per group was determined based on our preliminary experiments, where the convulsive dose of bupivacaine was 7.6 ± 1.1 mg/kg. Assuming a type I error protection of 0.05 and a power of 0.80 to detect a 20 % increase in the convulsive dose by lipid emulsion, eight animals were required in each group. All values are expressed as mean ± standard deviation (SD). Statistical analysis was performed using Sigma Stat 3.0 (Systat Software Inc., Richmond, CA, USA). Differences in the dose of anesthetics for inducing convulsions and cardiac

arrest and in the plasma concentration of anesthetics at the onset of convulsions and cardiac arrest between the two groups were compared using Student's *t* test. Differences in blood gas, MAP, and HR during all experiments among the four groups were examined using two-way analysis of variance (ANOVA) for repeated measures; subsequently, we examined the differences in MAP and HR within the same study groups at baseline, before bupivacaine or levobupivacaine infusion, and at the onset of convulsions using Tukey's test. Values were considered significant when $p < 0.05$.

Results

There were no intra- or intergroup differences in blood gas data among groups during all experiments (Table 1). There were no differences in either MAP or HR at baseline among the four groups (Table 2), and they did not change before bupivacaine or levobupivacaine infusion compared with baseline in either group. MAP significantly increased and HR significantly decreased at the onset of convulsions compared with baseline in all groups ($p < 0.01$ for all), with no differences among the four groups.

Tonic–clonic movements of extremities occurred simultaneously with the appearance of spike waves on EEG. Bupivacaine doses for inducing convulsions and cardiac arrest in the LB group (8.8 ± 1.7 and 10.2 ± 1.5 mg/kg, respectively) were significantly larger than those in the CB group (5.9 ± 1.1 and 7.1 ± 1.3 mg/kg, respectively, $p < 0.001$ for both, Fig. 1). Levobupivacaine doses for inducing convulsions and cardiac arrest in the LL group (10.0 ± 2.0 and 13.7 ± 3.6 mg/kg, respectively) were also significantly larger than those in the CL group (7.7 ± 1.6 and 9.4 ± 2.4 mg/kg, $p = 0.03$ and 0.02, respectively). These doses in the CL group were significantly larger than bupivacaine in the CB group ($p = 0.02$ and 0.04, respectively).

Plasma concentrations of bupivacaine at the onset of convulsions and cardiac arrest in the LB group (12.9 ± 2.9 and 41.4 ± 5.2 μg/ml, respectively) were significantly higher than those in the CB group (7.9 ± 1.2 and 21.6 ± 3.3 μg/ml, respectively, $p < 0.001$ for both, Fig. 2). Plasma

Table 1 Blood gas data

Variables	CB group	LB group	CL group	LL group
pH				
Baseline	7.47 ± 0.03	7.43 ± 0.04	7.44 ± 0.03	7.44 ± 0.07
Before infusion	7.43 ± 0.03	7.47 ± 0.03	7.45 ± 0.03	7.45 ± 0.05
Convulsions	7.44 ± 0.03	7.47 ± 0.03	7.44 ± 0.04	7.47 ± 0.03
$PaCO_2$ (mmHg)				
Baseline	34.3 ± 3.5	35.5 ± 3.9	34.8 ± 3.8	32.4 ± 4.2
Before infusion	32.6 ± 4.0	33.7 ± 4.0	35.2 ± 3.0	35.2 ± 4.3
Convulsions	34.9 ± 2.0	33.1 ± 2.3	33.1 ± 2.8	35.3 ± 3.7
PaO_2 (mmHg)				
Baseline	93.6 ± 3.8	88.0 ± 5.6	93.1 ± 6.0	93.1 ± 5.1
Before infusion	92.6 ± 3.4	89.4 ± 4.9	91.9 ± 5.6	93.4 ± 4.9
Convulsions	90.1 ± 5.6	94.3 ± 3.7	93.2 ± 3.9	92.3 ± 4.5

Data are expressed as the mean ± standard deviation (SD) of eight experiments. There are no intra- or intergroup differences in pH, $PaCO_2$, or PaO_2

$PaCO_2$ partial pressure of carbon dioxide in arterial blood, *PaO_2* partial pressure of oxygen in arterial blood, *CB* control–bupivacaine, *LB* lipid–bupivacaine, *CL* control–levobupivacaine, *LL* lipid–levobupivacaine groups

Table 2 Hemodynamic parameters

Variables	CB group	LB group	CL group	LL group
Mean arterial pressure (mmHg)				
Baseline	115 ± 6	122 ± 10	113 ± 6	117 ± 8
Before infusion	112 ± 8	112 ± 7	108 ± 8	115 ± 6
Convulsions	179 ± 19**	171 ± 16**	163 ± 15**	170 ± 13**
Heart rate (bpm)				
Baseline	397 ± 25	385 ± 46	381 ± 47	385 ± 28
Before infusion	389 ± 53	407 ± 33	396 ± 50	393 ± 46
Heart rate (bpm)	270 ± 41**	241 ± 37**	266 ± 47**	262 ± 39**

Data are expressed as the mean ± standard deviation (SD) of eight experiments. There are no differences among the four groups. MAP was significantly increased ($p < 0.01$) and HR significantly decreased ($p < 0.01$) at the onset of convulsions compared with baseline in all groups

CB control–bupivacaine, *LB* lipid–bupivacaine, *CL* control–levobupivacaine, *LL* lipid–levobupivacaine groups, *MAP* mean arterial blood pressure, *HR* heart rate

** $p < 0.01$ compared with baseline

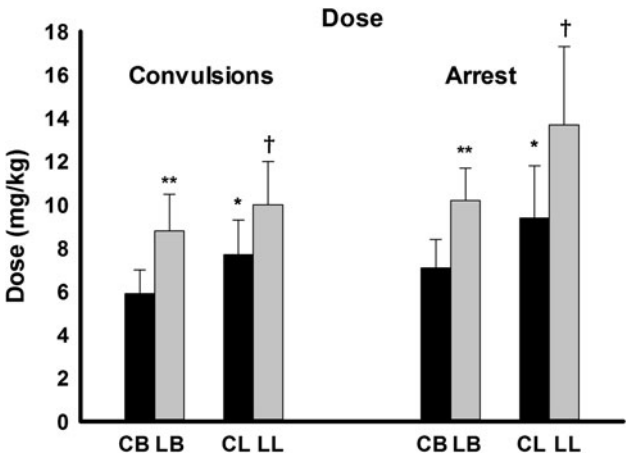

Fig. 1 Bupivacaine and levobupivacaine doses for inducing convulsions and cardiac arrest after pretreatment with saline [control–bupivacaine (CB) and control–levobupivacaine (CL) groups] or 20 % lipid emulsion [lipid–bupivacaine (LB) and lipid–levobupivacaine (LL) groups]. Data are expressed as mean ± standard deviation (SD) of eight experiments. *$p < 0.05$ and **$p < 0.01$ compared with the CB group. †$p < 0.05$ compared with the CL group

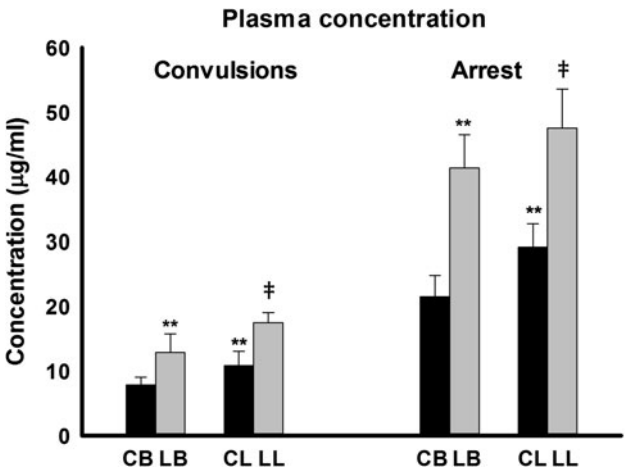

Fig. 2 Plasma concentrations of bupivacaine and levobupivacaine at the onset of convulsions and cardiac arrest after pretreatment with saline [control–bupivacaine (CB) and control–levobupivacaine (CL) groups] or 20 % lipid emulsion [lipid–bupivacaine (LB) and lipid–levobupivacaine (LL) groups]. Data are expressed as mean ± standard deviation (SD) of eight experiments. $^{**}p < 0.01$ compared with CB group. $^{\ddagger}p < 0.01$ compared with the CL group

concentrations of levobupivacaine at the onset of convulsions and cardiac arrest in the LL group (17.5 ± 1.5 and 47.6 ± 6.1 μg/ml, respectively) were also significantly higher than those in the CL group (10.9 ± 2.2 and 29.2 ± 3.5 μg/ml, respectively, $p < 0.001$ for both). These plasma concentrations in the CL group were also significantly higher than plasma concentrations of bupivacaine in the CB group ($p = 0.005$ and <0.001, respectively).

Discussion

In this study, pretreatment with lipid emulsion significantly increased the convulsive doses and threshold plasma concentrations of both bupivacaine and levobupivacaine for inducing convulsions. Bupivacaine doses necessary to induce convulsions without lipid emulsion were comparable with those reported in awake-animal models [13, 15]. CNS toxicity, manifested by excitation and convulsions, is directly related with cardiac toxicity and often precedes symptoms such as arrhythmia and bradycardia [6–9]. Convulsion suppression is suggested as a key in treating local-anesthetic-induced systemic toxicity, and benzodiazepines are recommended as the first-choice agents [16]. However, it is not clear whether benzodiazepines are effective for preventing the development of circulatory collapse following convulsions. On the other hand, successful prevention of cardiovascular collapse by suppressing convulsions with lipid emulsion in a patient receiving local anesthetics has been shown [8]. Despite this superior antidote effect of lipid emulsion, to our best knowledge, no studies have examined its effect on CNS toxicity of local anesthetics.

Pretreatment with lipid emulsion also increased the doses of local anesthetics necessary for inducing cardiac arrest and plasma concentrations at cardiac arrest, consistent with results shown by Weinberg et al. [2]. Notably, however, in our study, doses of bupivacaine necessary to induce asystole as well as plasma concentrations at that time with/without lipid emulsion were less than half of the values reported by Weinberg et al. [2]. General anesthesia with isoflurane as well as prevention of hypoxia by mechanical ventilation with 100 % oxygen under endotracheal intubation would be responsible for the remarkably larger doses necessary to induce cardiac arrest in their study. Previous studies also show the protective effect of propofol and sevoflurane against bupivacaine-induced bradycardia and cardiac arrest [3].

In contrast to numerous clinical reports describing effective treatment of refractory cardiac arrest resulting from an overdose of various local anesthetics, such as bupivacaine, levobupivacaine, ropivacaine, and lidocaine by lipid emulsion [5–9], most animal experiments have focused on bupivacaine; few reports examined the effect of lipid emulsion on the toxic effect of other local anesthetics [11]. Our study shows that lipid emulsion decreased the systemic toxicity of levobupivacaine and bupivacaine, results supported by previous studies showing high solubility and binding of both anesthetics to lipid emulsion [10] and reversal of levobupivacaine-induced vasodilation in isolated rat aorta by lipid emulsion [11].

Several mechanisms have been suggested to explain the antidote effect of lipid emulsion [1]. Currently, the most favored theoretical mechanism is the "lipid sink" theory, whereby partitioning of lipophilic local anesthetics into lipid emulsion rapidly decreases their concentrations in tissue and plasma [2]. An alternative mechanism is the lipid flux theory [17], whereby lipid emulsion improves the energy supply to the heart, which is impaired by bupivacaine. According to this theory, lipid emulsion increases the influx of acylcarnitine—an essential compound for the metabolism of fatty acid required to produce energy for cardiac contraction—into mitochondria in the cardiac muscle. Whereas the lipid sink theory is able to explain the effect of lipid emulsion on the CNS and on cardiac toxicity, the lipid flux theory is specific to the heart, and its applicability to the CNS is unclear. In our study threshold plasma concentrations for inducing both CNS and cardiac toxicity were increased by lipid emulsion, which could be explained by the lipid sink theory, although the content of local anesthetics in these organs or the fraction unbound to either lipid or protein was not measured and the mechanism of lipid rescue remains unclear.

There are several limitations to our study. First, we examined only the effect of pretreatment with lipid emulsion and not its effect on treating CNS toxicity or on resuscitation after cardiac arrest. Second, although our experimental model is similar to clinical condition of patients receiving neural blockade, hypoxia induced by convulsions besides cardiac toxicity of local anesthetics would contribute to cardiac arrest.

In conclusion we show that lipid emulsion decreased CNS and cardiac toxicity of both bupivacaine and levobupivacaine.

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