# Lipid Resuscitation of Bupivacaine Toxicity

## *Long-chain Triglyceride Emulsion Provides Benefits over Long- and Medium-chain Triglyceride Emulsion*

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## ABSTRACT

**Background:** The superiority of Intralipid, a long-chain triglyceride (LCT) emulsion *versus* Lipovenoes, a long- and medium-chain triglyceride (LCT/MCT) emulsion, in reversing local anesthetic-induced cardiac arrest is poorly defined and needs to be determined.

**Methods:** The study included two parts: in experiment A, bupivacaine (20 mg/kg) was injected to produce asystole. Either Intralipid 20% (LCT group, n = 30) or Lipovenoes 20% (LCT/MCT group, n = 30) with epinephrine was infused immediately. Return of spontaneous circulation and recurrence of asystole after resuscitation were recorded. In experiment B, 80 rats using the same model and resuscitation protocol were divided into 10 groups: $LCT_0$, $LCT_{15}$, $LCT_{30}$, $LCT_{60}$, and $LCT_{120}$ and $LCT/MCT_0$, $LCT/MCT_{15}$, $LCT/MCT_{30}$, $LCT/MCT_{60}$, and $LCT/MCT_{120}$ (n = 8 each; the subscripts represent respective observation period). $LCT_{15}$–$LCT_{120}$ and $LCT/MCT_{15}$–$LCT/MCT_{120}$ groups received Intralipid 20% or Lipovenoes 20%, respectively. Plasma and myocardial bupivacaine and triglyceride concentrations, as well as myocardial bioenergetics, were determined.

**Results:** In experiment A, 24 rats in LCT group and 23 in LCT/MCT group achieved return of spontaneous circulation ($P = 0.754$); among them, 2 (8.3%) and 8 (34.8%) rats suffered a repeated asystole, respectively ($P = 0.027$). In experiment B, plasma and myocardial bupivacaine concentrations in $LCT_{15}$ and $LCT_{60}$ groups were lower than $LCT/MCT_{15}$ and $LCT/MCT_{60}$ groups, respectively. Furthermore, the plasma bupivacaine level in $LCT/MCT_{60}$ group was higher than $LCT/MCT_{30}$ group ($P = 0.003$).

**Conclusions:** LCT emulsion may be superior to LCT/MCT emulsion in treating bupivacaine-related cardiotoxicity as it was associated with fewer recurrences of asystole after resuscitation and lower myocardial bupivacaine concentrations.

### What We Already Know about This Topic

- Intravenous lipid emulsion is an effective treatment for local anesthetic-induced cardiovascular collapse
- Intravenous lipid emulsions contain long-chain triglycerides and long- and medium-chain triglycerides, components which are essential to its benefits in this setting

### What This Article Tells Us That Is New

- Long-chain triglyceride emulsion in rats with local anesthetic-induced cardiovascular collapse was superior to long- and medium-chain triglyceride emulsion and was associated with fewer recurrences of asystole after resuscitation and lower myocardial bupivacaine concentrations

FOLLOWING the serendipitous discovery of lipid rescue from local anesthetic-induced cardiac arrest by Weinberg *et al.*[1] in 1998, a growing body of reported data has provided evidence for the efficacy of lipid rescue in both laboratory models and humans, thereby establishing intravenous lipid emulsion as an effective treatment for local anesthetic-induced cardiovascular collapse.[2–3]

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Intravenous lipid emulsions containing long-chain triglycerides (LCTs) and long- and medium-chain triglycerides (LCTs/MCTs) are widely used in clinical scenarios. The two formulation types of commercially available lipid emulsions most commonly used are Intralipid (a 100% LCT emulsion; Huarui Pharmaceuticals Co., Ltd., Wuxi, China) and Lipovenoes (a 50:50 wt:wt mixed LCT/MCT emulsion; Huarui Pharmaceuticals Co., Ltd.). Intralipid contains 100% soybean oil 200 g/L, phosphatide 12 g/L, and glycerol 22 g/L, whereas Lipovenoes consists of a mixture of 50% soybean oil and 50% medium chain triglycerides 200 g/L, phosphatide 12 g/L, and glycerol 25 g/L.

Most of the published work to date has focused on LCT emulsions, Intralipid[4–9] and Liposyn III.[10] Recently there have been several reported cases of successful resuscitation using Medialipid 20%,[11–12] which contains 50/50 LCTs/MCTs. Clear recommendations on lipid therapy have now been published by both the Association of Anesthetists of Great Britain and Ireland and American Society of Regional Anesthesia and Pain Medicine.[2] Specifically, both professional societies currently recommend administration of an initial bolus dose of 1.5 ml/kg followed by an infusion of 15 $ml \cdot kg^{-1} \cdot h^{-1}$. If cardiovascular stability is not attained or deteriorates, rebolusing at 5-min intervals and doubling the infusion rate should be considered. Nevertheless, the recommendations do not address the choice of lipid emulsions.

The exact mechanism of lipid rescue remains unclear. Currently, the most favored theoretical mechanism is the "lipid sink" theory, in which the lipophilic local anesthetics are bound by lipid, thus reducing tissue content of the toxin.[1] An alternative mechanism is the "lipid flux" theory, whereby lipid emulsions supply the mitochondria with sufficient substrate to enable energy production, which counters the impaired fatty acid delivery caused by local anesthetics.[13] Furthermore, there are reports of differences in speed of hydrolysis and oxidation,[14] the binding affinity of bupivacaine,[15] the production of ketone bodies,[16] and metabolic pathways of transport and utilization[17] between LCT and LCT/MCT emulsions. Some authors also believe that they may have different effects on bupivacaine-induced cardiotoxicity because of their different particle sizes (430 nm *vs.* 280 nm, respectively).[18]

We hypothesize that LCT emulsions provide similar return of spontaneous circulation (ROSC), fewer recurrences of asystole after ROSC, and lower myocardial bupivacaine concentrations than did LCT/MCT emulsions. Accordingly, a double-blind prospective randomized animal study was undertaken with the primary aim of establishing the rate of mortality after ROSC following administration of LCT (Intralipid, 20%) or LCT/MCT (Lipovenoes, 20%) emulsions in an established rat model of bupivacaine cardiotoxicity, and secondarily, to determine the rate of ROSC and survival at an endpoint of 120 min. In addition, plasma and myocardial bupivacaine and triglyceride concentrations, as well as myocardial bioenergetics, were determined to account for the possible difference between them.

## Materials and Methods

Two experiments were performed. In experiment A, the effects of two commercially available lipid emulsions of different compositions, on the recovery from bupivacaine toxicity in a cardiac rat model, were evaluated. In experiment B, plasma and cardiac tissue bupivacaine concentrations under the two lipid emulsion treatments at predetermined time levels were analyzed, as were triglyceride levels and myocardial bioenergetics. All studies were conducted with the approval of Wenzhou Medical College's Animal Care and Use Committee (Wenzhou, China).

### Experimental Animals

Healthy male Sprague-Dawley rats, 7 to 8 weeks old, were purchased from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China), and housed in plastic cages (4 rats per cage) at the animal center of Wenzhou Medical College in a standard 12-h reverse day/night cycle at an ambient temperature of 26°C. The rats had an *ad libitum* diet of stock laboratory diet (Beijing Keao-Xieli Feedstuff Co., Ltd., Beijing, China) and tap water. They had a recovery period of 1 to 2 weeks following transportation to Wenzhou Medical College before experiments. After the animals had exhibited adequate weight gain, rats age 8–10 weeks, weighing 300–400 g, were entered into subsequent studies.

### Experimental Model

The rats were fasted for 12 h before the experiments, with free access to water. All experiments commenced at or before 9 AM. On the day of the experiment, rats were anesthetized with an intraperitoneal injection of chloral hydrate (350 mg/kg). They were then intubated *via* tracheotomy and mechanically ventilated with 1% to 2% sevoflurane in 100% oxygen, using a rodent volume-controlled ventilator (HX-300, TME Technology Co., Ltd., Chengdu, China): tidal volume = 8 ml/kg, respiratory rate = 75–80 breaths/min, inspiratory: expiratory ratio = 2:3. The right internal jugular vein, the left femoral vein, and femoral artery were cannulated. Body temperature was maintained at 38° to 39°C with a heating lamp held at a safe distance. Electrocardiography, using three subcutaneous needle electrodes, and the arterial pressure were recorded continuously throughout the duration of the protocol by a MedLab data archiving and retrieval system using U/4C051 (Nanjing Medease Science and Technology Co., Ltd., Jiangsu, China). After completion of invasive procedures, all animals were then allowed to stabilize for 15 min at 0.5% sevoflurane and 100% oxygen, after which baseline heart rate, mean arterial pressure, and rate-pressure product (RPP) were recorded.

### Bupivacaine Arrest and Resuscitation Protocol

At the end of the stabilization period, sevoflurane was discontinued and immediately thereafter 20 mg/kg bupivacaine

hydrochloride (Sigma-Aldrich Co., St. Louis, MO) was injected as an intravenous bolus over 20 s *via* the venous cannula in the right femoral vein. The dose of 20 mg/kg was elected based on the work of Weinberg *et al.*[6] All rats developed asystole by the end of the bupivacaine infusion, and this was taken as time zero.

Cardiopulmonary resuscitation with mechanical ventilation and manually performed external chest compressions (approximately 30% anterior-posterior chest diameter at 200 compressions/min) were instituted immediately upon the onset of cardiac arrest (time zero). At the same time, animals were entered in a lipid infusion protocol as determined by prior randomization, and received Intralipid 20% for the LCT group, or Lipovenoes 20% for the LCT/MCT group, as a 5 ml/kg bolus over 30 s followed by a continuous infusion of 1.0 $ml \cdot kg^{-1} \cdot min^{-1}$ for 3 min, *via* the right femoral vein and the right internal jugular vein, respectively. This lipid therapy regimen was based on the work of Di Gregorio *et al.*[9] and Hiller *et al.*[19] with some modifications to optimize recovery in this model of bupivacaine overdose. Total dosing of 8 ml/kg was elected in accordance with the clinical recommendation of Weinberg.[20–21] A bolus of 10 μg/kg epinephrine (Shanghai Harvest Pharmaceutical Co., Ltd., Shanghai, China) was then administered over the subsequent 5 s at 1-min intervals until ROSC, with a maximum cumulative dose of 100 μg/kg. Chest compressions were delivered until a native RPP more than 20% of baseline value for 1 min or longer, which was our criterion for ROSC.[9] All chest compressions were stopped at the 15-min point regardless of RPP, and subjects were evaluated for sustained or nonsustained recovery until the 20-min point. Ventilation with 100% oxygen was continued throughout the 20 min of resuscitation efforts. Cardiopulmonary resuscitation was not recommenced after initial ROSC in animals subsequently developing a second period of cardiac arrest. All intravenous fluids were prepared and preheated to 37°C before infusion by personnel that did not participate in resuscitation efforts. Coordinated resuscitation efforts with clear division of roles were conducted according to the recommendation of Smith[22] with the following modifications: (1) provider one initiated specific lipid therapy and epinephrine administration, (2) provider two performed external chest compressions, and (3) provider three managed the airway and assessed native RPP during resuscitation. The two types of lipid emulsions have the same visual appearance, and all providers were blinded regarding the group.

### *Experiment A*

**Grouping of Animals and Setup.** Sixty rats were randomly allocated by the random table method before the study into LCT group and LCT/MCT group, with 30 animals in each group. The animal experimental procedures were conducted using the aforementioned bupivacaine arrest and resuscitation protocol. LCT and LCT/MCT groups received Intralipid 20% or Lipovenoes 20%, respectively. All surviving rats in both groups were observed for 120 min.

**Data Acquisition.** ROSC and rats surviving at 120 min were recorded. We then calculated the rate of ROSC, survival at 120 min, and mortality after resuscitation (rate of ROSC = number of rats displaying ROSC/total number of rats; survival at 120 min = number of rats survived at 120-min/total number of rats; mortality after resuscitation = cases of repeated asystole after ROSC/cases of initial ROSC). We also continuously recorded the systolic blood pressure, mean arterial pressure, heart rate, and RPP until the termination of the observation period, at which time we calculated the RPP recovery ratio (RPP recovery ratio = RPP of survival rats at specific time points/baseline RPP value). Time to first heartbeat, time to ROSC, and the cumulative dose of epinephrine were recorded.

### *Experiment B*

**Grouping of Animals and Setup.** A second group of 80 rats were assigned to 10 groups (eight animals per group) based on differing observation time using the random table method. The groups were defined as $LCT_0$, $LCT_{15}$, $LCT_{30}$, $LCT_{60}$, and $LCT_{120}$ and $LCT/MCT_0$, $LCT/MCT_{15}$, $LCT/MCT_{30}$, $LCT/MCT_{60}$, and $LCT/MCT_{120}$. After asystole, $LCT_{15}$–$LCT_{120}$ groups and $LCT/MCT_{15}$–$LCT/MCT_{120}$ groups received Intralipid 20% or Lipovenoes 20%, respectively. Monitoring and evaluation of hemodynamic status and airway management were conducted continuously during cardiopulmonary resuscitation and throughout respective observation period. At the end of the observation period, all the surviving animals in $LCT_{15}$–$LCT_{120}$ and $LCT/MCT_{15}$–$LCT/MCT_{120}$ groups were killed with cervical dislocation and a 4 ml blood sample was immediately taken by needle aspiration of the cardiac apex. The same sampling method was used for all animals in $LCT_0$ and $LCT/MCT_0$ groups upon the onset of cardiac arrest (without lipid emulsions infusion). The blood plasma was separated by centrifugation (for 10 min at 3,000 revolutions per minute) and cardiac tissues were rinsed to remove any residual blood. Finally, all samples were then stored at −70°C until analysis was performed.

**Data Acquisition.** ROSC and rat survival to the end of their respective observation periods were recorded. For all groups, the plasma and cardiac tissue bupivacaine concentrations were analyzed similar to previous reports[23] from this laboratory by high-performance liquid chromatography/mass spectrometry. Cardiac tissue sample preparation for triglyceride determination was conducted using the Song[24] procedure. Both plasma and tissue samples were then examined by a fully automatic Architect C 8000 Analyzer (Abbott Laboratories, Chicago, IL). Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate from heart tissue were measured by high-performance liquid chromatography. Sample preparation was conducted using the Stehr[25] procedure. Measurements were performed on an

Agilent 1100 series high-performance liquid chromatography (Agilent Technologies, Inc., Santa Clara, CA) equipped with UV-visible detection (wavelength range from 190 to 600 nm). Separations were carried out on a Hypersil ODS-2 column (200 × 4.6 mm, 5 μm; Dalian Elite Analytical Instrument Co., Ltd., Dalian, China) at 30°C. The mobile phase was a mixture of eluent A and B (pH = 6.0, 99:1, v:v) at a flow rate of 1.0 ml/min. Eluent A was phosphate buffer (12 mM $Na_2HPO_4$ and 88 mM $NaH_2PO_4$, pH = 6.0) and eluent B was a mixture of 85% eluent A and 15% acetonitrile. An injection volume of 20 μl was used applying a 7725i Rheodyne manual injector (Agilent Technologies, Inc.) for both standard samples and tissue extract samples. The detection wavelength was set at 254 nm. Equally treated external standards of known concentrations were used to check retention times and to permit sample quantification based on the analysis of peak area. The adenylate energy charge was calculated according to the formula: energy charge = (ATP + 0.5 × ADP) × (ATP + ADP + adenosine monophosphate)$^{-1}$.

### Statistical Analysis

Power analysis was based on results of preliminary data comparing mortality after ROSC between groupings. In our preliminary study, 16 rats received lipid emulsions immediately after bupivacaine-induced asystole (n = 8 for mIntralipid treatment; n = 8 for Lipovenoes treatment). One of seven (14.3%) animals displaying initial ROSC developed a second cardiac arrest after intralipid administration, whereas 3 of 6 (50%) survivors suffered a repeated asystole after Lipovenoes administration. Sample size calculations showed that a chi-square test with a type I error (two-sided) of 0.05 will have 88% power to detect a 35.7% difference in mortality after ROSC between the two groups, when the sample size in each group is 29. To account for potential attrition, we enrolled 30 rats per group.

Considering a survival of 75% (6 of 8) at 120-min endpoint for intralipid treatment and 37.5% (3 of 8) for Lipovenoes treatment shown in the preliminary experiment, eight rats were enrolled in each group so as to avoid a small number of survivors, which may statistically lead to inaccurate results obtained from determination of myocardial bupivacaine and triglyceride levels.

Data were tested for normal distribution using the Shapiro–Wilk test and the Kolmogorov–Smirnov test. Continuous variables were presented using means and SDs or medians and interquartile values, and in the case of categorical variables, frequencies were used. In experiment A, values for baseline parameters, times to ROSC, and times to first heartbeat were compared by Student *t* test, and the cumulative epinephrine dose was analyzed using the Wilcoxon and Mann–Whitney U test. Dichotomous outcomes were analyzed with Fisher exact test. Continuous hemodynamic variables in animals surviving at 120 min were compared across time by two-way repeated measures ANOVA, with Bonferroni correction posttesting when significance was achieved ($P < 0.05$). Time-to-event data were studied with Kaplan–Meier analysis and compared using the log-rank test. In experiment B, baseline parameters were analyzed by one-way ANOVA. The comparison of bupivacaine, triglyceride, and adenine-nucleotides between groups with the same observation time was evaluated by Student *t* test. As primary outcome variables, bupivacaine among the same emulsion-treated groups was compared using an initial Kruskal–Wallis H-test. If significance was achieved for differences, subanalysis according to the Bonferroni correction was applied. All statistical analysis was performed with SPSS for Windows

**Table 1.** Baseline Values of Weight and Haemodynamic Metrics for LCT and LCT/MCT Groups in Experiment A and for LCT and LCT/MCT Groups in Experiment B

| Experiment | Group | n | Weight (g) | MAP (mmHg) | Heart Rate (Beat/Min) | RPP (mmHg × Beat/Min) |
|---|---|---|---|---|---|---|
| Experiment A | LCT | 30 | 347 ± 25 | 112 ± 13 | 413 ± 38 | 54223 ± 7,648 |
| — | LCT/MCT | 30 | 339 ± 20 | 114 ± 12 | 418 ± 59 | 54248 ± 7,429 |
| *P* value | — | — | 0.16 | 0.62 | 0.68 | 0.99 |
| Experiment B | $LCT_0$ | 8 | 350 ± 26 | 120 ± 14 | 411 ± 55 | 55069 ± 8,971 |
| — | $LCT_{15}$ | 8 | 350 ± 22 | 122 ± 14 | 411 ± 57 | 57332 ± 9,398 |
| — | $LCT_{30}$ | 8 | 345 ± 28 | 111 ± 11 | 403 ± 26 | 50899 ± 4,660 |
| — | $LCT_{60}$ | 8 | 350 ± 27 | 114 ± 12 | 413 ± 24 | 51978 ± 8,006 |
| — | $LCT_{120}$ | 8 | 351 ± 26 | 113 ± 12 | 428 ± 33 | 56818 ± 5,703 |
| — | $LCT/MCT_0$ | 8 | 337 ± 20 | 122 ± 9 | 405 ± 38 | 55084 ± 6,818 |
| — | $LCT/MCT_{15}$ | 8 | 340 ± 20 | 123 ± 12 | 403 ± 43 | 55360 ± 7,794 |
| — | $LCT/MCT_{30}$ | 8 | 337 ± 30 | 114 ± 12 | 403 ± 28 | 53417 ± 6,652 |
| — | $LCT/MCT_{60}$ | 8 | 335 ± 13 | 113 ± 10 | 408 ± 27 | 52637 ± 3,795 |
| — | $LCT/MCT_{120}$ | 8 | 338 ± 19 | 112 ± 5 | 453 ± 61 | 55456 ± 7,328 |
| *P* value | — | — | 0.79 | 0.07 | 0.34 | 0.71 |

Values are given as mean ± SD. Baseline values for major parameters showed no difference between LCT and LCT/MCT groups in experiment A and among 10 groups in experiment B. Subscript numbers in experiment B represent respective observation period.
LCT = long-chain triglyceride; MAP = mean artery pressure; MCT = medium-chain triglyceride; RPP = rate-pressure product (systolic blood pressure × heart rate).

**Table 2.** Resuscitation Outcomes for LCT and LCT/MCT Groups in Experiment A

| Characteristics | LCT (n = 30) | LCT/MCT (n = 30) | *P* Value |
|---|---|---|---|
| Rate of ROSC (%) | 80.0% (24) | 76.7% (23) | 0.754 |
| Survival to 120-min (%) | 73.3% (22) | 50.0% (15) | 0.063 |
| Mortality after resuscitation (%) | 8.3% (2) | 34.8% (8) | 0.027 |
| Times to ROSC (s) | 245 ± 54 | 223 ± 83 | 0.264 |
| Times to first heartbeat (s) | 100 ± 36 | 79 ± 45 | 0.086 |
| Epinephrine cumulative dose (μg/kg) | 40 (30, 62.5) | 50 (30, 92.5) | 0.529 |

Normal data are given as mean ± SD, whereas non-normal data are expressed as median and interquartile values.
LCT = long-chain triglyceride; MCT = medium-chain triglyceride; ROSC = return of spontaneous circulation.

(version 14.0; SPSS, Chicago, IL). Statistical significance was considered as $P < 0.05$.

## Results

### *Experiment A*

**Characteristics of Study Subjects.** Body weight, baseline values of mean arterial blood pressure, heart rate, or RPP before intervention did not differ significantly between LCT and LCT/MCT groups (table 1).

**Resuscitation Outcomes.** The LCT and LCT/MCT groups displayed similar ROSC (24 and 23 animals respectively, 80.0% *vs.* 76.7%, $P = 0.754$). More importantly, 8 of 23 animals displaying initial ROSC in LCT/MCT group subsequently developed a second period of intractable cardiac arrest compared with only 2 of 24 in LCT group (8.3% *vs.* 34.8%, $P = 0.027$). Almost all of repeated cardiac arrest occurred in the period from 10 to 45 min. Therefore, 22 animals in LCT group survived to protocol termination, compared with 15 animals in LCT/MCT group (73.3% *vs.* 50.0%, $P = 0.063$). There were no significant differences between LCT and LCT/MCT groups in respect to time to ROSC ($P = 0.264$), time to first heartbeat ($P = 0.086$), and cumulative dose of epinephrine ($P = 0.529$) (table 2). Death distribution and Kaplan–Meier survival curves of rats that exhibited ROSC in LCT and LCT/MCT groups are presented in figure 1. The median survival time of animals displaying initial ROSC was 120 (interquartile, 0) min and 120 (interquartile, 91) min in LCT and LCT/MCT groups, respectively (chi-square test = 4.643, $P = 0.031$).

**Hemodynamic Measures.** Hemodynamic parameters heart rate (fig. 2), systolic blood pressure (fig. 3), and RPP (fig. 4) in animals surviving to protocol termination are presented graphically. LCT emulsions produced superior conditions in terms of heart rate ($F = 22.69$, $P < 0.001$), systolic blood pressure ($F = 4.35$, $P = 0.044$), and RPP ($F = 17.87$, $P < 0.001$) than did LCT/MCT emulsions. The graphical disparity reported primarily occurred at the midpoint of the observation period, and the curves of both groups flatten out gradually in the later periods. LCT emulsions resulted in a more complete recovery of RPP at 15 ($P = 0.004$), 30 ($P = 0.001$), and 60 min ($P < 0.001$) than did LCT/MCT emulsions. RPP recovery ratio for the 90–120 min did not differ between the groups ($P > 0.05$) (fig. 5).

### *Experiment B*

**Characteristics of Study Subjects.** In experiment B, there were 6, 5, 6, and 6 surviving rats in $LCT_{15}$, $LCT_{30}$, $LCT_{60}$, and $LCT_{120}$ groups, respectively, and 4, 4, 4, and 5 in $LCT/MCT_{15}$, $LCT/MCT_{30}$, $LCT/MCT_{60}$, and $LCT/MCT_{120}$

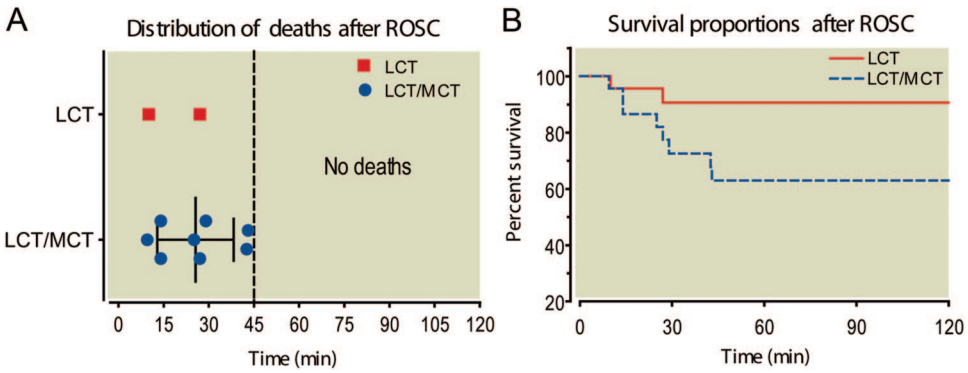


**Fig. 1.** Death distribution (*A*) and Kaplan–Meier survival curves (*B*) of rats exhibiting return of spontaneous circulation (ROSC) in long-chain triglyceride and long- and medium-chain triglyceride groups. (*A*) Eight of 23 animals displaying return of spontaneous circulation in the long- and medium-chain triglyceride group died at 25.5 ± 12.7 min (75% CI: 19.9, 31.1) after the onset of initial asystole, whereas 2 of 24 in the long-chain triglyceride group died at 10 min and 27 min, respectively. (*B*) The median survival time of rats exhibiting return of spontaneous circulation in the long-chain triglyceride group was longer than that in the long- and medium-chain triglyceride group (chi-square test = 4.643, $P = 0.031$). LCT = long-chain triglyceride; MCT = medium-chain triglyceride.

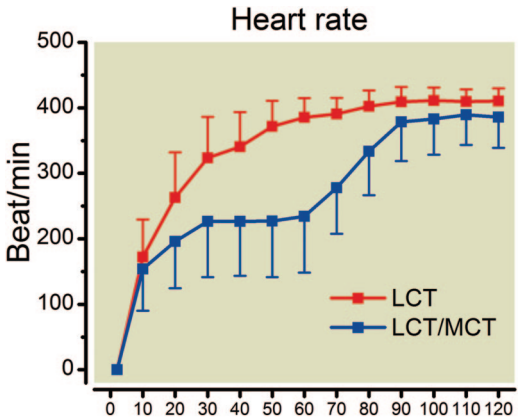


**Fig. 2.** Heart rate *versus* time for rats that survived to 120 min. Long-chain triglyceride emulsions produced superior heart rate recovery (F = 22.69, $P < 0.001$) than did long- and medium-chain triglyceride emulsions. N = 22 for long-chain triglyceride group and 15 for long- and medium-chain triglyceride group. Data are mean and error bars SD. LCT = long-chain triglyceride; MCT = medium-chain triglyceride.

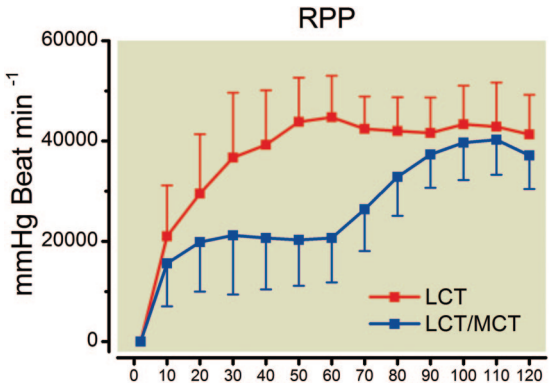


**Fig. 4.** Rate-pressure product *versus* time for rats that survived to 120 min. Long-chain triglyceride emulsions produced superior rate-pressure product recovery (F = 17.87, $P < 0.001$) than did long- and medium-chain triglyceride emulsions. N = 22 for long-chain triglyceride group and 15 for long- and medium-chain triglyceride group. Data are mean and error bars SD. LCT = long-chain triglyceride; MCT = medium-chain triglyceride; RPP = rate-pressure product.

groups, respectively. Baseline body weight and hemodynamic parameters (heart rate, mean arterial pressure, and RPP) were similar among the 10 groups (table 1).

**Bupivacaine Measurement.** The concentrations of bupivacaine in cardiac tissue and plasma are presented graphically in figure 6. Data indicate a lower bupivacaine level in the $LCT_{15}$ group (8.1 ± 2.2 μg/g; 14.1 ± 2.2 μg/ml; cardiac tissue content, plasma concentration, respectively) than the $LCT/MCT_{15}$ group (13.8 ± 1.7 μg/g; 29.7 ± 5.5 μg/ml), and in the $LCT_{60}$ group (2.0 ± 0.8 μg/g; 3.8 ± 1.8 μg/ml) than the $LCT/MCT_{60}$ group (5.9 ± 0.7 μg/g; 16.1 ± 1.8 μg/ml). Mean ± SD; $P < 0.05$ for all shown results. LCT emulsion-treated groups showed a monotonously decreased plasma bupivacaine, whereas LCT/MCT emulsion-treated groups exhibit an overall decline with an unexpected plasma concentration rebound that was 16.1 ± 1.8 μg/ml in the $LCT/MCT_{60}$ group and 8.3 ± 2.7 μg/ml in the $LCT/MCT_{30}$ group, $P = 0.003$.

**Triglyceride Level.** There was no difference in the triglyceride content of cardiac tissue between groups with the same observation time ($P > 0.05$) (fig. 6B). No difference in plasma triglyceride levels was found between $LCT_0$ and $LCT/MCT_0$, as well as between $LCT_{15}$ and $LCT/MCT_{15}$ groups ($P > 0.05$) (fig. 6D). There was a steady decline from the 15-min point ($LCT/MCT_{15}$) to the 120-min point ($LCT/MCT_{120}$). It was lower in the $LCT/MCT_{120}$ group as compared to the $LCT_{120}$ group ($P < 0.001$).

**Myocardial Bioenergetics.** No major differences in the myocardial bioenergetics between groups with the same observation time was observed, including ATP, ADP, adenosine monophosphate, and energy charge (fig. 7).

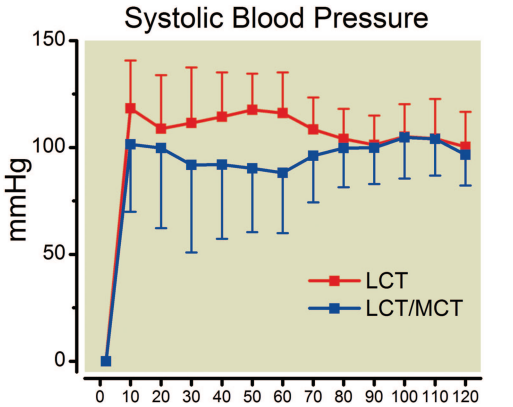


**Fig. 3.** Systolic blood pressure *versus* time for rats that survived to 120 min. Long-chain triglyceride emulsions produced superior systolic blood pressure recovery (F = 4.35, $P = 0.044$) than did long- and medium-chain triglyceride emulsions. N = 22 for long-chain triglyceride group and 15 for long- and medium-chain triglyceride group. Data are mean and error bars SD. LCT = long-chain triglyceride; MCT = medium-chain triglyceride.

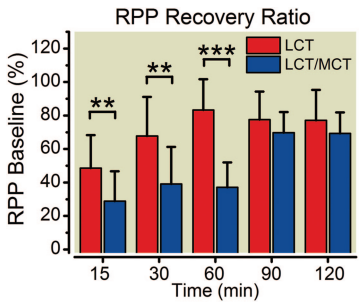


**Fig. 5.** Effects of lipid emulsions on rate-pressure product recovery ratio at 15, 30, 60, 90, and 120 min induced by 20 ml/kg intravenous bupivacaine. Asterisks represent significance of differences between long-chain triglyceride (n = 22) and long- and medium-chain triglyceride (n = 15) groups at 15, 30, and 60 min. ** $P < 0.01$; *** $P < 0.001$. Data are mean and error bars SD. LCT = long-chain triglyceride; MCT = medium-chain triglyceride; RPP = rate-pressure product.

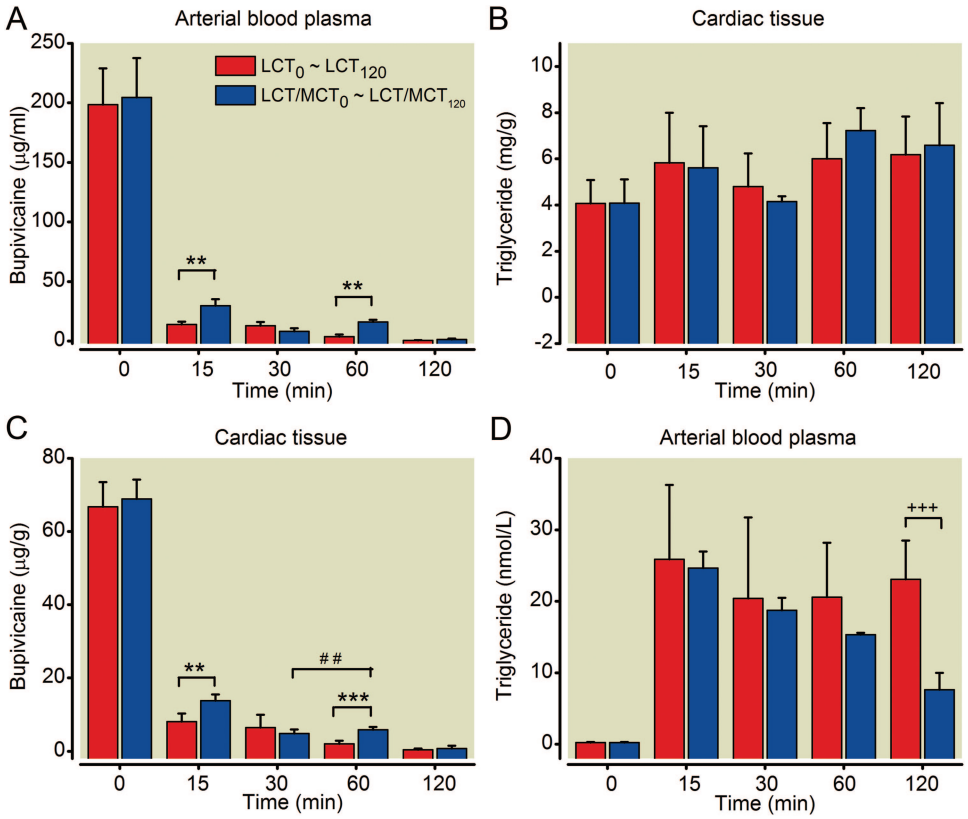


**Fig. 6.** Bupivacaine and triglyceride level of surviving rats before and after lipid emulsions rescue treatment. The cardiac tissue and plasma concentration of bupivacaine (*A*, *C*) and triglyceride (*B*, *D*) were compared between groups with the same observation time. ** $P < 0.01$ between long-chain triglyceride 15-min group and long- and medium-chain triglyceride 15-min group; *** $P < 0.001$ between long-chain triglyceride 60-min group and long- and medium-chain triglyceride 60-min group; ## $P < 0.01$ between long- and medium-chain triglyceride 30-min group and long- and medium-chain triglyceride 60-min group; +++ $P < 0.001$ between long-chain triglyceride 120-min group and long- and medium-chain triglyceride 120-min group. N = 8, 6, 5, 6, and 6 in long-chain triglyceride 0–120-min groups and 8, 4, 4, 4, and 5 in long- and medium-chain triglyceride 0–120-min groups, respectively. Data are mean and error bars SD. LCT = long-chain triglyceride; MCT = medium-chain triglyceride.

## Discussion

This rodent model demonstrated that both LCT and LCT/MCT emulsions reversed the cardiac arrest induced by the intravenous injection of 20 mg/kg bupivacaine. They produced the relatively similar rates of ROSC ($P = 0.754$). There was, however, a significant difference in mortality after resuscitation was observed between the LCT and LCT/MCT groups (8.3% *vs.* 34.8%, $P = 0.027$). With respect to survival at 120 min, it approached significance ($P = 0.063$); we had powered the study to find a 35.7% difference ($\beta = .88$), but we only found a 23.3% difference. Thus, the null hypothesis regarding survival may not have been rejected (potential Type II error). In addition, the RPP recovery ratio during the period from 15 to 60 min were also much higher in LCT group. The concentrations of bupivacaine in cardiac tissue and arterial plasma were significantly lower in $LCT_{15}$ and $LCT_{60}$ groups than those in $LCT/MCT_{15}$ and $LCT/MCT_{60}$ groups, respectively.

Epinephrine is a first-line drug for treating cardiac arrest because its $\alpha$-agonist activity increases diastolic pressure and coronary perfusion pressure.[26] Recent work by Harvey *et al.*[5] demonstrated that epinephrine was necessary for return of spontaneous circulation in lipid-based resuscitation from local anesthetic-induced cardiac arrest. This finding is consistent with our previous work in the isolated rat heart model of bupivacaine toxicity, wherein lipid emulsion combined with epinephrine produced superior coronary flow and RPP value than resuscitation with lipid emulsion alone.[27]

The results in experiment B indicate that both treatments resulted in a sharp decline in total myocardial bupivacaine concentration during the initial 30 min after asystole, a time period during which cardiac tissue bupivacaine content decreased by 90.4% after LCT emulsion treatment and by 93.0% after LCT/MCT emulsion treatment. Overall, the similar measurements at 0 min and 30 min suggested they may exert their curative effects in a 30-min time period. Thereafter, it was observed that the plasma bupivacaine concentration dramatically increased in the $LCT/MCT_{60}$ group relative to the $LCT/MCT_{30}$ group, whereas it exhibited an extended decline after LCT emulsion treatment, which indi-

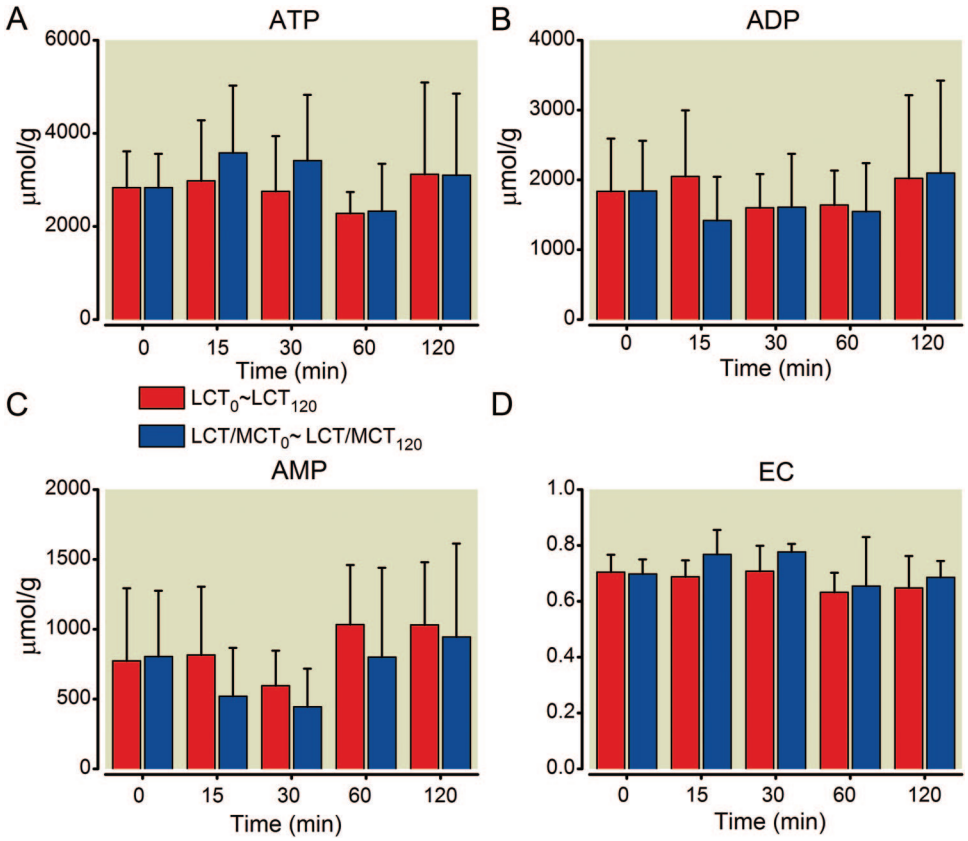


**Fig. 7.** Myocardial bioenergetics measurements. (*A*) Adenosine triphosphate (ATP), (*B*) adenosine diphosphate (ADP), (*C*) adenosine monophosphate (AMP), and (*D*) energy charge (EC) [(ATP + 0.5 × ADP) × (ATP + ADP + AMP)$^{-1}$] revealed no significant differences between groups with the same observation time. N = 8, 6, 5, 6, and 6 in long-chain triglyceride 0–120-min groups and 8, 4, 4, 4, and 5 in long- and medium-chain triglyceride 0–120-min groups, respectively. Data are mean and error bars SD. LCT = long-chain triglyceride; MCT = medium-chain triglyceride.

cated a longer action time when LCT emulsion was compared to LCT/MCT emulsion. The half-life may have accounted for this observation: the estimated half-life was 33 min for LCTs and 17 min for MCTs,[28] for which clearing of the plasma LCT/MCT was completed earlier than LCT.[14] More studies suggest that a substantial amount of exogenous fatty acids reach both hepatic and nonhepatic tissues as intact triacylglycerol without preceding lipolysis in the plasma compartment.[29] On the basis of the "lipid sink" hypothesis and the profound liver-targeting property for lipid emulsions,[30–31] it can be hypothesized that the redistribution and metabolism of lipid emulsion upon hemodynamic recovery may have allowed subsequent hepatic release of bupivacaine that was incorporated within the core of LCT/MCT lipid emulsion. This hypothesis may explain the distinct effects in hemodynamic parameters during the period from 15 to 60 min as well as the deaths after resuscitation concentrated in 10–45 min in LCT/MCT group in experiment A. However, we did not measure the hepatic bupivacaine content, and this is a possibility for future research.

In addition to the above, the high lipid solubility of local anesthetics and the difference in the high binding capacity of the lipid emulsions explained their distinct efficacy. Mazoit *et al.*,[15] using the "shake-flask" method *in vitro* to measure the extent of emulsification of lipophilic local anesthetics, found that the extent of local anesthetic binding to Intralipid 20% was roughly 2.5 times more than Medialipide 20% (B-Braun, Boulogne, France). This indicated that LCT emulsions were more efficient than LCT/MCT emulsions in binding to long-acting local anesthetics. Compared with the LCT/MCT$_{15}$ group, the lower cardiac tissue bupivacaine content in the LCT$_{15}$ group may chiefly result from the better capacity of LCT lipid emulsion to bind bupivacaine and an ensuing liver-targeting transport of lipid-soluble bupivacaine. The Lipovenoes in our protocol is similar to the Medialipid used by Mazoit *et al.*, as both of them are lipid emulsions containing equal proportions of LCTs and MCTs (although made by different manufacturers).

Under normal aerobic conditions, fatty acids are the preferred substrate for cardiomyocyte, generating about 80–90% of ATP.[32] Lipid emulsions could theoretically increase intracellular fatty acid content, by impeding a local anesthetic's inhibition of acylcarnitine and ATP synthesis[7,13] in the cardiomyocyte, thereby improving ATP production cardiac function. Carnitine is needed to transport long-chain fatty

acids into the mitochondria.[33] Conversely, medium-chain fatty acids can enter the mitochondria by simple diffusion independent of the carnitine enzyme system.[34] Thus, specificity for rapid oxidation, without deposition in fat stores,[14] carnitine-independent transport to the site of oxidation, and the ability to supply energy with higher ketone bodies make MCTs theoretically a preferable choice from an energy-related perspective. Unfortunately, the theoretical benefits of LCT/MCT lipid emulsions are not borne out in practice. No difference in ATP, ADP, adenosine monophosphate, and energy charge throughout the duration of the protocol was observed in experiment B, which was similar with that of Stehr's research data from an isolated rat heart model.[25]

Our investigation has several limitations. First, our small animal model necessitated that each animal in experiment B had to be sacrificed at its assigned sampling time point. Thus, repeated measures for each animal were not possible. Nonetheless, we stringently controlled the animal profiles including the animal source, strain, sex, age, body weight, housing condition, and experimental environment to reduce the error as much as possible. Second, the dosing protocols of key study drugs (lipid emulsions, epinephrine) that we used may not be the optimal. However, the regimen we used was found in preliminary experiments used to optimize recovery in this model of bupivacaine intoxication. The effective reversal of cardiovascular symptoms and our highly successful resuscitation suggest validation of our model. A third limitation regards the power of our study. While we were powered to find a 35.7% difference, we, nonetheless, still present a solid basis for further experimentation and discussion, especially in light of the fact that our work supports Weinberg's[6,7,9,19] and confirms his studies.

In conclusion, LCT emulsion may be the choice of lipid resuscitation from bupivacaine toxicity as it provided less mortality after resuscitation, lower myocardial bupivacaine concentrations, and superior hemodynamic recovery than did LCT/MCT emulsion in an intact rat model of bupivacaine-induced cardiovascular collapse, which we would attribute to the differences of "lipid sink" effect, rather than to the "lipid flux" theory. LCT emulsion's superiorities showed in the present study may further strengthen the guidelines for the management of severe local anesthetic toxicity with lipid emulsion. Further research may be warranted regarding the comparison of LCT and LCT/MCT emulsions in another humanized animal model, although it is unlikely that LCT/MCT emulsions will show any benefit over LCT emulsions.

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