Lipid emulsion pretreatment has different effects on mepivacaine and bupivacaine cardiac toxicity in an isolated rat heart model†

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Editor’s key points

- Lipid emulsions are used clinically to treat local anaesthetic (LA) toxicity, but their ability to reduce toxicity prophylactically is poorly studied.
- Using an isolated rat heart model, pretreatment with clinical doses of a lipid emulsion delayed onset of asystole and speeded recovery after bupivacaine but not mepivacaine.
- Efficacy of lipid emulsion pretreatment appears to be greater for more lipophilic LAs in this ex vivo animal model.

Background. The use of lipid emulsions to reduce cardiac toxicity of local anaesthetics (LAs) has shown success in experimental studies and some clinical cases, and thus has been implemented in clinical practice. However, lipid treatment is usually given after the occurrence of neurological or cardiovascular symptoms of systemic intoxication. The aim of this study was to determine if pretreatment with lipid emulsion reduces cardiac toxicity produced by bupivacaine or mepivacaine.

Methods. Isolated rat hearts were perfused with or without lipid emulsion (0.25 ml kg⁻¹ min⁻¹) before administration of equipotent doses of bupivacaine (250 μM) or mepivacaine (1000 μM). Haemodynamic parameters and times from start of perfusion LA to a 1 min period of asystole and recovery were determined.

Results. Pretreatment with lipid emulsion extended the time until occurrence of asystole and decreased times to recovery in bupivacaine-induced cardiac toxicity but not in mepivacaine-induced cardiac toxicity compared with control. Lipid pretreatment impaired rate–pressure product recovery in mepivacaine-intoxicated hearts.

Conclusions. This study confirms that pretreatment with a lipid emulsion reduces cardiac toxicity of LAs. The efficacy of pretreatment with lipid emulsion was LA-dependent, so pharmacokinetic properties, such as lipophilicity, might influence the effects of lipid emulsion pretreatment.

Keywords: bupivacaine; cardiac arrest; fat emulsion; heart; mepivacaine

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In 1998, Weinberg and colleagues¹ reported that either pretreatment or resuscitation with a lipid emulsion substantially shifted the dose–response to bupivacaine-induced asystole in rats. Since then, the administration of lipid emulsions to treat local anaesthetic (LA)-induced cardiac toxicity has been extensively investigated.²⁴ In addition, benefits of lipid emulsion therapy observed in clinical cases have led to implementation of lipid therapy as a rescue medication for LA systemic toxicity (LAST).⁵ ⁶ Furthermore, there is an increasing number of case reports of beneficial effects of lipid therapy on cardiac toxicity induced by drugs other than LAs.⁸ ⁹ However, the optimal time of administration, dose, etc., of lipid administration remain controversial.¹⁰¹²

The ‘lipid sink’ effect, which posits the existence of a lipid compartment into which lipophilic LAs such as bupivacaine concentrate, might explain the lipid rescue of cardotoxicity. Our group has recently shown that there is a lipophilicity-dependent effect of lipid emulsion therapy using an isolated rat heart model.⁵ In this study, administration of a lipid emulsion improved recovery from bupivacaine-induced cardiac toxicity but not from mepivacaine-induced cardiac arrest. These findings are supported by Laine and colleagues¹³ who showed in an in vitro model that the ability of a lipid to bind LAs depends on the LA lipophilicity. In contrast to less lipophilic LAs, bupivacaine demonstrates concentration-dependent lipid-binding properties.¹³ Furthermore, Mazoit and colleagues have shown that the solubility and capacity of a lipid emulsion to bind potent, long-acting LAs depends on the amount of long-chain triglycerides (LCTs) present in the emulsion and the lipophilicity of the LA. Therefore, bupivacaine and levobupivacaine seem to be cleared more rapidly in response to lipid emulsions than ropivacaine, which offers a possible explanation for the clinical effects of lipid emulsion therapy.¹⁶

Currently, lipid emulsions are administered as a therapy for LAST and its neurological and cardiovascular symptoms. However, clinical failures using lipid rescue raises the concern that timing of lipid emulsion infusion may play an important role; therefore, a pretreatment strategy might have more...
beneficial effects.15 16 Charbonneau and colleagues17 reported a case in which they treated incipient symptoms of mepivacaine intoxication, which seemed to prevent progression to more severe toxicity. However, experimental studies and other clinical experiences concerning this aspect of lipid treatment are absent, especially regarding their different effects and physicochemical properties. In the present study, isolated rat hearts were pretreated with lipid emulsions to assess their effects on bupivacaine- and mepivacaine-induced cardiac toxicity. We hypothesized that the physicochemical properties of LAs result in different effects of lipid pretreatment.

Methods

Preparation and measurements

After obtaining approval from the Institutional Animal Care Committee of the University of Regensburg (Regensburg, Germany), 100 mg kg⁻¹ ketamine, 2.5–5 mg kg⁻¹ xylazine hydrochloride, and 1000 units of heparin were injected intraperitoneally into each of 32 Wistar rats [average weight, 228 (8) g]. After decapitation and sternotomy, the aorta was rapidly cannulated and the heart was excised by retrograde continuous aortic root perfusion using cold, oxygenated, modified Krebs–Ringer solution (KRS) equilibrated with 95% oxygen and 5% carbon dioxide, and then the heart was transferred to a Langendorff apparatus (Hugo Sachs Electronic KG, March-Hugstetten, Germany) as previously reported.18 The KRS was filtered (5 μm pore size filter disk, Sigma-Aldrich®, Munich, Germany) in-line and was composed of the following: 140 mM Na⁺; 4.5 mM K⁺; 1.2 mM Mg²⁺; 2.5 mM Ca²⁺; 134 mM Cl⁻; 15.5 mM HCO₃⁻; 1.2 mM H₂PO₄⁻; 0.05 mM EDTA; 11.5 mM glucose (all Sigma-Aldrich, Steinheim, Germany); 2 mM pyruvate (AppliChem, Darmstadt, Germany); 10 mM mannitol (Thermo-Fisher, Schwerte, Germany); and 5 units litre⁻¹ insulin (B.Braun Ratiopharm, Melsungen, Germany). Perfusion was maintained at a flow of 12 ml min⁻¹ by a roller pump (Ismattec®, Glattbrugg, Switzerland). The temperature of the perfusate and the heart was maintained at 36.9 (0.2) °C. Oxygen tension and electrolytes in the coronary inflow and outflow vessels were measured using a self-calibrating blood gas analyzer (Gem Premier 4000, IL GmbH, Germany). The mean aortic inflow pH, CO₂ tension (Pco₂), and O₂ tension (Po₂) were 7.4 (0.03), 4.53 (0.3), and 78 (2.7) kPa, respectively.

A thin, saline-filled latex balloon (Hugo Sachs Electronic KG) was inserted into the left ventricle and was attached to a metal cannula. The cannula was then connected to a pressure transducer (Isotec®, Hugo Sachs Electronic KG) to measure changes in isovolumetric systolic left ventricular pressure (LVP). Balloon volume was adjusted to maintain an initial diastolic LVP of 0 mm Hg during the control period so that any increase in diastolic LVP reflected an increase in left ventricular wall stiffness or diastolic contracture. Once the balloon volume was set, the volume remained constant throughout the experiment. Two pairs of bipolar silver electrodes (Teflon-coated silver, diameter 125 μm, Cooner Wire, Chatsworth, LA, USA) were attached to the right atrium and the pulmonary conus to monitor intracardiac electrical activity from which spontaneous sinoatrial and ventricular rates were measured as previously described.19 Heart rate, LVP, coronary inflow, and pressure were continuously monitored, displayed on a screen, and digitally recorded on a hard drive every 10 s, and the rate–pressure product [RPP = (left ventricular systolic pressure – left ventricular diastolic pressure) × heart rate] was calculated.

Protocol

After stabilization for 10 min, baseline values were recorded and hearts were randomly assigned to one of the 2 × 2 groups (8 hearts each). In Group 1, the perfusion solution consisted of KRS alone (control group 1: Bupi) or KRS with lipid emulsion (treatment group 1: Bupi+Lipid) at a dose of 0.25 ml kg⁻¹ min⁻¹ (Lipofundin© MCT 20%, B. Braun, Melsungen, Germany) for 10 min until reaching a haemodynamic steady state (Fig. 1), at which point baseline values were recorded again. The same protocol was used with mepivacaine (control group 2: Mepi; treatment group 2: Mepi+Lipid; 8 hearts each; Fig. 1).

The decision to use a 10 min period before starting LA infusion was based on two criteria: first, to ensure the steady state, and secondly, to simulate the clinical approach when performing regional anaesthesia. We used this lipid dose as it provided reproducible and significant effects in previous LA-induced cardiac toxicity studies using an isolated heart model.15 20 Furthermore, the dose corresponds to the recommended therapeutic dose from the recently published European Resuscitation Council Guidelines for Resuscitation.21 Because we used a flow-controlled setting to deliver KRS, our calculated lipid concentration was ~0.5%. The lipid emulsion was added using a perfusion pump (Braun® perfusor compact, Melsungen, Germany) positioned directly above the heart. Thereafter, hearts were perfused with KRS-containing ‘equipotent’ doses (based on their reported cardiac toxicity) of bupivacaine (250 μM) or mepivacaine (1000 μM; both from AstraZeneca®, Södertälje, Sweden) until a 1 min period of asystole was observed.5 22 Thereafter, lipid and LA infusion were stopped. The time that elapsed and haemodynamic changes that occurred from the introduction of LA drug until a 1 min period of asystole (T1), the time from recovery until the first heart beat (T2), and the time 60 min after recovery (T3) were measured in each group (Fig. 1).

Statistical analysis

Baseline T1 and T2 values were compared using an unpaired Student t-test, and corresponding effect sizes were calculated according to Cohen’s d. For comparisons between treatment groups (Bupi vs Bupi+Lipid and Mepi vs Mepi+Lipid) at different time points in the asystolic and recovery phases, linear mixed models were used. The mean values and standard errors of the mean (SEM) were used as parameter estimates and adjusted with post hoc pairwise comparisons by the Bonferroni method. In all mixed models, time was used as a repeated effect, and the correlation structure between time points was specified as autoregressive. P < 0.05 was considered significant. The statistical software used to conduct analyses was SAS 9.3 (SAS Institute Inc., Cary, NC, USA). All data in the text, tables, and figures are displayed as the mean (SEM).
Results

Baseline values for each heart at steady state and before starting LA infusion showed no differences between the groups (Table 1). Times from the start of perfusion to observation of a 1 min period of asystole were 248 (12), 244 (13), 269 (9), and 242 (16) s for Bupi, Mepi, Bupi + Lipid, and Mepi + Lipid, respectively. Times to occurrence of asystole for Bupi and Bupi + Lipid were statistically significantly different (Table 1). Times to recurrence of heart rhythm after asystole, as measured by the first heart beat observed after asystole, showed no statistical differences among groups (Table 1). The RPP showed a decelerated decrease and accelerated recovery with lipid infusion in bupivacaine-intoxicated hearts (Fig. 2). However, in the lipid-pretreated hearts intoxicated with mepivacaine, the recovery of RPP was significantly impaired (Fig. 3).

Discussion

Our results show that pretreatment with lipid infusion decreases cardiac toxicity in bupivacaine- but not mepivacaine-intoxicated hearts. Lipid emulsion significantly delayed occurrence of asystole and accelerated recovery of RPP, the product of left ventricular function and heart rate, in bupivacaine-intoxicated hearts. In mepivacaine-intoxicated hearts, lipid emulsions had no beneficial effect and even impaired recovery of RPP.

Weinberg and colleagues showed that pretreatment with a lipid emulsion substantially shifted the dose–response to bupivacaine-induced asystole in a whole animal model. With increasing lipid concentration, an increased amount of bupivacaine (up to almost four times) was necessary to induce asystole.1 Our study supports the basic observation that lipid emulsions reduce the cardiac toxicity of bupivacaine at an even lower dose of lipid. Weinberg and colleagues used 3 ml kg⁻¹ min⁻¹, whereas we used a lipid dose based on recent resuscitation guidelines.21 Additionally, our study protocol analysed recovery of cardiac performance and showed a marked decrease.

Table 1 Baseline values, intoxication (with bupivacaine and mepivacaine), and recovery. Baseline values (HR, heart rate; RPP, rate-pressure product), time until occurrence of a 1 min period of asystole (T1), and time until recurrence of the first heart beat after a 1 min period of asystole (T2) in hearts pretreated with lipid emulsion (protocol groups: Bupivacaine + Lipid (Bupi+Lipid) and Mepivacaine + Lipid (Mepi+Lipid)) or without lipid emulsion (control groups: Bupivacaine (Bupi) and Mepivacaine (Mepi)). The effect sizes were calculated according to Cohen’s d. Data are presented as mean (SEM). *P < 0.05

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<tr>
<th></th>
<th>Bupi</th>
<th>Bupi + Lipid</th>
<th>P-value</th>
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<tr>
<td>HR (beats min⁻¹)</td>
<td>316 (11)</td>
<td>310 (8)</td>
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<td>RPP (mm Hg beats min⁻¹)</td>
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<td>36 (13)</td>
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<tr>
<td>Time until asystole (T1)</td>
<td>248 (12)</td>
<td>269 (9)*</td>
<td>0.04</td>
<td>1.0</td>
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<td>Time until recovery (T2)</td>
<td>439 (24)</td>
<td>395 (28)</td>
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<table>
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<tr>
<th></th>
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<tr>
<td>HR (beats min⁻¹)</td>
<td>309 (10)</td>
<td>300 (11)</td>
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<td>RPP (mm Hg beats min⁻¹)</td>
<td>35 (12)</td>
<td>35 (13)</td>
<td>0.8</td>
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<tr>
<td>Time until asystole (T1)</td>
<td>244 (13)</td>
<td>242 (16)</td>
<td>0.33</td>
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<tr>
<td>Time until recovery (T2)</td>
<td>259 (7)</td>
<td>301 (26)</td>
<td>0.08</td>
<td>0.8</td>
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Fig 1 Study protocol: steady state, stabilization phase of heart function; lipid pretreatment, phase of lipid infusion; T1, time until the occurrence of a 1 min period of asystole; T2, time until the recurrence of the first heart beat after asystole; and T3, 60 min recovery phase. Bupi, bupivacaine; Mepi, mepivacaine; Lipid, lipid emulsion; LA, local anaesthetic.
**Fig 2** RPP of bupivacaine perfusion under control conditions (Bupi) or with lipid pretreatment (Bupi + Lipid) from the start until end of the experiment. Bupi or Bupi + Lipid were infused until the first minute after the start of asystole. The RPP for each minute is presented as mean (SE). The adjusted P-values were not significant. *P < 0.05.

**Fig 3** RPP of mepivacaine perfusion under control conditions (Mepi) or with lipid pretreatment (Mepi + Lipid) from the start until end of the experiment. Mepi or Mepi + Lipid were infused until the first minute after the start of asystole. The RPP for each minute is presented as mean (SE). The adjusted P-values were significant for minutes 6 and 7. *P < 0.05.
Lipid emulsion pretreatment and local anaesthetic toxicity

improvement in RPP after recovery from bupivacaine-induced asystole. This effect might be easily explained by the ‘lipid sink’ theory, in which lipids absorb excess bupivacaine to reduce its total concentration in the heart and therefore accelerate recovery. However, the time until recovery was not significantly reduced which might be explained by a possibly insufficient lack of power.

Lipid emulsion might also have led to a direct effect on recovery by influencing metabolism or a mechanical performance of the left ventricle. Indeed, Stehr and colleagues demonstrated a direct inotropic effect soon after initiation of lipid infusion. In a recent study from the same group, bupivacaine-intoxicated hearts showed mitochondrial swelling and reduction in cellular metabolism accompanied by a negative inotropic effect. The reduction in cellular metabolism was reversible in the presence of fatty acids, which could at least partly explain the inotropic effect of lipid emulsions. Furthermore, administration of medium-chain triglycerol-containing emulsions led to improvement of post-ischaemic cardiac function in isolated hearts and alterations of cation fluxes in ventricular cardiomyocytes, suggesting that lipids have direct effects on cardiomyocytes. Taken together, these data suggest that there could be other mechanisms besides the ‘lipid sink’ in ameliorating LA-induced cardiac toxicity. With respect to the effect observed in the recovery phase in our results, it is possible that lipids have direct and long-lasting effects on cardiomyocytes.

Weinberg and colleagues hypothesized that lipid emulsions form a lipid compartment in the blood, in which lipophilic substances such as bupivacaine dissolve, reducing bupivacaine concentration in the intoxicated heart. In this respect, the binding capacity of the lipid emulsion and the solubility of LAs depend on characteristics of the lipid emulsions (e.g. the fraction and concentration of LCTs) and of the LAs. Highly lipophilic, bupivacaine and levobupivacaine clear more rapidly than the less lipophilic ropivacaine. Furthermore, by increasing the lipid concentration of a lipid emulsion, more bupivacaine becomes trapped, whereas less lipophilic LAs, such as lidocaine or prilocaine, are not affected by lipid content. These results have been partially confirmed in studies using isolated and intact rodent models. Therefore, the lipid sink effect is dependent on the lipophilic properties of the LAs as well. We showed that a lipid emulsion improves recovery from bupivacaine-induced cardiac toxicity but not from ropivacaine- or mepivacaine-induced cardiac arrest. However, lipid pretreatment effects with less lipophilic LAs, such as mepivacaine, were not available.

Our study is the first to describe the a priori infusion of lipid in mepivacaine-intoxicated hearts. Lipid pretreatment showed no beneficial effects and even led to impaired recovery of RPP after asystole. These results are basically identical to observations from other studies showing that lipid treatment does not improve recovery of heart rhythm or RPP after asystole in an isolated heart model, and confirms in vitro results in which less ‘lipid binding’ with less lipophilic LAs was observed. Owing to the less lipophilic nature of mepivacaine, it might not have been effectively dissolved in the lipid emulsion. Additionally, this might have led to impaired wash-out of mepivacaine, resulting in delayed RPP recovery. In our view, this supports the lipid sink hypothesis and its dependence on the physicochemical properties of the intoxicating drug. Interestingly, these results do not support direct effects of lipid on metabolism or mechanical performance as described above. Further experimental efforts are necessary here.

We did not test our hypothesis with different types of lipid solutions or concentrations. Our primary goal was to use a ‘safe’ dose for lipid pretreatment, which is necessary in a clinical setting because the incidence of LAST is low. Therefore, we used 0.25 ml kg⁻¹ min⁻¹, a dose that is recommended for the treatment of LAST and comparable with the dose recommended for safe administration of lipid emulsion as nutrition in intensive care. Other experimental studies using similar doses in isolated heart models have shown reproducible and significant effects in bupivacaine-induced cardiac toxicity. High doses of lipid (e.g. 3 ml kg⁻¹ min⁻¹ for 5 min) can substantially shift the dose–response to bupivacaine-induced asystole in a rodent model. Additionally, high doses of lipid seem to be superior to vasopressin or epinephrine in animal models of resuscitation from bupivacaine-induced cardiac arrest. Therefore, one could argue that higher lipid doses might have altered our results, especially, as case reports have described success with lipid treatment with less lipophilic LA-induced cardiac toxicity. However, these high doses would require infusion of ~1 litre of undiluted lipid emulsion for a human being, which would be too high for a pretreatment strategy.

Another lipid solution may have shown different results. There are different solutions on the market, such as Intralipid®, an LCT emulsion containing 100% soybean oil at a concentration of 200 g litre⁻¹, or Lipofundin®, a long- and medium-chain triglyceride (LCT/MCT) emulsion mixture of 50% soybean oil and 50% MCTs at concentrations of 200 g litre⁻¹. Li and colleagues showed that LCT emulsions might be superior to LCT/MCT emulsions in treating bupivacaine-related cardiotoxicity. With the use of LCT, fewer recurrences of asystole after resuscitation and lower myocardial bupivacaine concentrations were observed. However, time to return of spontaneous circulation, cumulative epinephrine dose, and survival up to 120 min were not different between the LCT and LCT/MCT groups. As our study protocol lasted <90 min, we doubt that a different solution would have modified our basic findings. Furthermore, any concentration- or solution-dependent effects of lipid therapy would not influence the fundamental correlation between lipophilic substances and the ‘lipid sink’ effect. A potential limitation of the current study is the use of an ex vivo model. Therefore, care must be taken in directly comparing our results with those derived from other in vivo experiments or clinical reports because pharmacokinetic characteristics can differ. In contrast to other models, an isolated heart model allows investigation of the effects of lipid pretreatment without influence from metabolic or systemic factors. Therefore, our model offers the best opportunity to study direct cardiac effects. Another limitation is that recovery of heart function (T3) might have been affected by the time of LA perfusion in...
T1. In this context, longer T1 means greater delivery of LA, which in turn leads to higher accumulation of LA in the heart. However, in the Mepi and Mepi + Lipid groups, the time to asystole was not different. In contrast, Bupi + Lipid had a longer infusion time compared with its control. Nevertheless, recovery in the lipid-pretreated group was accelerated compared with controls.

In conclusion, our data support the reduction in cardiac toxicity of bupivacaine by pretreatment with lipid emulsion. Furthermore, pretreatment has a positive effect on recovery from cardiac toxicity. These effects might be especially important in intoxication with highly lipophilic drugs, such as bupivacaine. With less lipophilic LAs, such as mepivacaine, no beneficial effects were observed with lipid pretreatment. These results suggest that the ‘lipid sink’ effect is strongly dependent on the lipophilicity of the LA.

Authors’ contributions
C.A., B.K., and Y.A.Z. proposed the study and performed preliminary experiments. C.A. and B.K. contributed equally. B.K. and H.B. performed experiments. M.G. coordinated laboratory support. C.A., Y.A.Z., and W.Z. were responsible for writing the paper. Y.A.Z., C.H.W., and B.M.G. edited the manuscript and added important comments to the paper. All authors read and approved the final manuscript.

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Declaration of interest
None declared.

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