Hemodynamic Effects of Intravenous, High-Dose Lipid Emulsion With and Without Metoprolol Infusion in Healthy Volunteers: A Randomized Clinical Trial

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In a double-blinded, randomized, crossover trial, we investigated the hemodynamic effects of high-dose intravenous lipid emulsion (ILE) with/without metoprolol. Ten healthy volunteers each completed 4 trial days (placebo + ILE; metoprolol + placebo; metoprolol + ILE; placebo + placebo) in random order. Metoprolol was administered as an initial bolus (10 mg), followed by an infusion (50 mg) from 5 to 30 minutes. ILE was administered as a bolus at 12.5 minutes (2.5 mL/kg), followed by a 15-minute infusion (0.25 mL/kg per minute). On metoprolol + ILE days (compared with metoprolol + placebo) after 120 minutes, mean heart rates were significantly higher (difference, 5.5 beats per minute (bpm); 95% confidence interval (CI), 3.0–8.1 bpm; $P < 0.001$), and average relative cardiac output was higher (difference, 10 percentage points; 95% CI, 5–15 percentage points; $P < 0.001$). The hemodynamic effect of ILE developed gradually. ILE had no effect on plasma metoprolol or major adverse events. In conclusion, high-dose ILE has relatively marginal and delayed hemodynamic effects that may have limited clinical relevance in the short-term clinical toxicological setting.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ The clinical evidence concerning efficacy and safety of high-dose intravenous lipid emulsion (ILE)—a therapy increasingly used in the management of drug overdoses—is limited.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ We assessed whether ≈400 mL of ILE would affect hemodynamic end points (heart rate, blood pressure, stroke volume, and cardiac output) alone and during cardiac beta[1] blockade with 60 mg metoprolol.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ We observed that ILE led to small, gradually increasing heart rate, stroke volume, and cardiac output in healthy volunteers. Effects were comparable with and without metoprolol pretreatment. No effects of ILE on plasma metoprolol concentrations were observed.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Our observations (i) suggest that interventions with a more rapid onset of action would be preferable in cases of metoprolol (i.e., β-blocker) overdose and (ii) contrast to the rapid effects of ILE often reported in case reports and may thus question the use of ILE for metoprolol poisoning.

Administration of intravenous lipid emulsion (ILE) for hemodynamic stabilization in conditions of systemic toxicity caused by local anesthetics is recommended by anesthetic and toxicological scientific societies.[1–3] Recommendations are primarily based on findings from animal studies and clinical case reports.[4] Proposed mechanisms behind possible hemodynamic stabilizing effects of ILE include binding of the drug in the plasma lipid phase, which may provide a beneficial redistribution effect,[5] volume effects, and cardiotonic effects.[6] The American College of Medical Toxicology also regards ILE as

a potential option for severe poisonings with lipophilic drugs if patients are hemodynamically unstable and not responsive to standard resuscitation measures.[3] Thus, use of ILE has expanded to severe poisonings with other drugs than local anesthetics (e.g., β-blockers) in which beneficial effects also have been reported in animal models[7–9] and clinical case reports.[10–14] However, the overall quality of evidence concerning use of ILE for other indications than local anesthetic poisoning is low,[2,15] and systematic reviews of case reports of poisonings find contradictory interpretations of available case data.[16,17] At the same time, adverse effects of ILE such as lung injury, pancreatitis, and interference with laboratory tests have been observed.[10,18,19]

Our objectives were therefore to (i) investigate in healthy adult volunteers whether high-dose ILE in combination with or without an infusion of the β-blocker metoprolol exerted effects on heart rate, cardiac contractility, blood pressure, and cardiac conduction compared with saline placebo and (ii) explore whether ILE affected metoprolol plasma concentrations and was associated with adverse effects.

RESULTS

Participant flow is presented in **Figure 1**. Inclusion took place from September to December 2016. Ten enrolled participants completed 4 trial days each between September 2016 and March 2017. Baseline participant demographics are presented in **Table 1**.

Heart rate

Difference between metoprolol + placebo and metoprolol + ILE at 120 minutes (90 minutes after ILE infusion stop) in heart rate was 5.5 beats per minute (bpm) (95% confidence interval (CI), 3.0–8.1 bpm; $P < 0.001$) (**Figure 2a, b**). On both metoprolol days, we observed mean heart rate reductions of 5–6 bpm (95% CI, −2.5 to −8.6 bpm; $P < 0.01$) from baseline to the 12.5-minute timepoint (start of ILE/placebo administration). On days with metoprolol + ILE, the average heart rate was higher than on metoprolol + placebo days from 60 to 120 minutes. On the metoprolol + placebo day, the average heart rate remained below baseline (−6.6 bpm; 95% CI, −8.8 to −4.3 bpm; $P = 0.01$) at 120 minutes (from 57 bpm at baseline). On days with placebo + ILE, the average heart rate was 4.5 bpm (95% CI, 2.0–7.0 bpm; $P < 0.01$) higher than on placebo + placebo days at 120 minutes. There were no statistically significant effects of ILE compared with placebo before the 60-minute timepoint (30 minutes after the end of infusions) or on heart rate incremental area under the curve (iAUC) from start of ILE/placebo bolus (**Figure 2c**). The final population-level pharmacokinetic–pharmacodynamic (PK–PD) model for heart rate predicted the observed changes in heart rate (i.e., an initial ILE-induced heart rate decrease of 3.3 bpm; 95% CI, 3.7–3.9 bpm), followed by a linear increase in heart rate. Furthermore, the model revealed a 3% (95% CI, 2.7–3.1%) decrease in maximal inhibitory effect of metoprolol. All PK–PD data are presented in **Methods S1**, PK–PD.
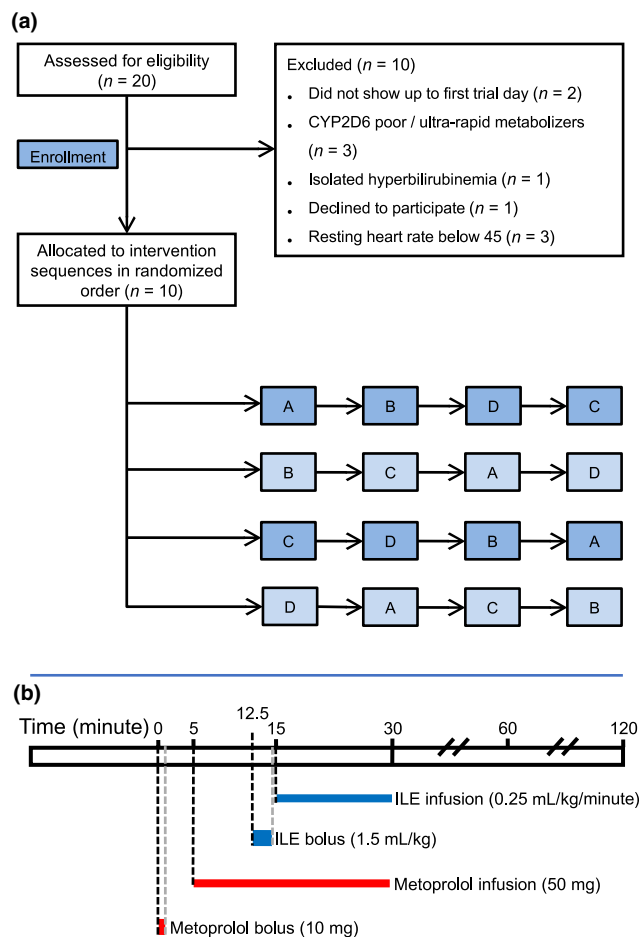

(a)

(b)

Figure 1 (**a**) Trial flow diagram; screening, enrollment, randomization, and intervention sequences. Interventions: A, placebo + ILE; B, metoprolol + placebo; C, metoprolol + ILE; D, placebo + placebo. (**b**) Interventions on trial days. Black vertical dotted lines: start of infusions. Gray vertical dotted lines: stop of infusions. Metoprolol (0.5 mg/mL) or placebo was administered as a 20-mL bolus over no more than 1 minute, followed by a 4 mL/minute infusion (thin red line) from time 5 to 30 minutes. ILE or placebo was administered as a 1.5 mL/kg bolus over 1–2 minutes at time 12.5 minutes (blue line), followed by a 0.25 mL/kg per minute bolus from time 15 to 30 minutes (thin blue line). ILE, intravenous lipid emulsion.

Blood pressure

Between-day differences in systolic blood pressure values were not statistically significant at any timepoint (mean differences, −7 to 4 mmHg; 95% CI, −14 to 11 mmHg; $P = 0.06$–0.8) (**Figure 3a, b**). Systolic blood pressure iAUC from start of ILE/placebo bolus was larger on days with metoprolol + ILE compared with metoprolol + placebo days (566 mmHg × minutes; 95% CI, 296–836 mmHg × minutes; $P = 0.01$) (**Figure 3c**). A maximum reduction from baseline in the average systolic blood pressure of 9 mmHg (95% CI, 4–14 mmHg; $P = 0.02$) was observed at the 50-minute timepoint on the metoprolol + placebo day (from 137 mmHg at baseline) (**Figure 3**). The ILE bolus increased mean arterial pressure (MAP) up to 4 mmHg (95% CI, 1–7 mmHg; $P = 0.02$) (**Figure S1**, Mean Arterial Pressure) and diastolic blood pressure up to 3 mmHg on days with concomitant metoprolol infusion at 20 and 30 minutes (**Figure S2**, Diastolic

Table 1 Baseline characteristics of study participants

Characteristics	Mean	SD	Range
Age (years)	24.2	3.7	20–30
Weight (kg)	73.4	8.0	65–88
Height (m)	1.8	0.1	1.75–1.90
Body mass index (kg/m^2)	22.2	1.9	20–25
Systolic blood pressure (mmHg)	135.8	8.9	113–159
Diastolic blood pressure (mmHg)	68.3	4.0	58–78
Mean blood pressure (mmHg)	87.7	4.5	76–97
Heart rate (bpm)	62.8	7.6	49–70
Alanine aminotransferase (U/L)	20.1	7.1	11–32
Albumin (g/L)	41.4	3.9	37–50
Aspartate aminotransferase (U/L)	19.3	4.6	12–28
Alkaline phosphatase (U/L)	59.2	11.2	50–84
Bilirubin (µmol/L)	10.1	3.5	5–14
Estimated average glucose (mmol/L)[a]	5.4	0.3	4.9–5.7
Glomerular filtration rate (mL/minute)	> 90		
Hemoglobin (mmol/L)	9.1	0.6	8.4–10
HbA1c (mmol/mol)	31.3	2.2	27–33
HbA1c (%)	5.0	0.2	4.7–5.2
High-density lipoprotein (mmol/L)	1.4	0.4	0.97–1.87
Low-density lipoprotein (mmol/L)	2.1	0.5	1.2–2.8
Triglycerides (mmol/L)	1.0	0.7	0.49–2.87
Cholesterol (mmol/L)	3.9	0.6	3.3–5.1
Potassium (mmol/L)	4.0	0.4	3.6–4.8
Sodium (mmol/L)	140.9	1.2	139–143
Creatinine (µmol/L)	82.5	12.8	71–108
Urinary albumin (mg/L)	5.5	3.7	< 3–13
Urinary creatinine (mmol/L)	17.1	9.9	3.8–40.5

Bpm, beats per minute; HbA1c, hemoglobin A1c.
[a]Estimated average glucose = 0.1455 · HbA1c (mmol/mol) + 0.8285.

Blood Pressure). There was higher MAP on ILE days compared with placebo when looking at iAUC (313 mmHg × minutes; 95% CI, 71–554 mmHg × minutes; $P = 0.02$) (**Figure S1**, Mean Arterial Pressure).

Stroke volume and cardiac output
On metoprolol + ILE days, there was a four–percentage point higher relative stroke volume compared with metoprolol + placebo at 120 minutes (95% CI, 1–7 percentage points; $P = 0.01$). On the metoprolol + placebo day, the relative stroke volume reduction from baseline to 120 minutes was 7% (95% CI, −10%

to −4%; $P = 0.02$) (**Figure 4a**). During metoprolol infusion, the ILE bolus led to a decrease in cardiac output at 20 minutes of 6 percentage points (95% CI, 1–11 percentage points; $P = 0.02$). On days with metoprolol + ILE, the relative cardiac output was higher compared with metoprolol + placebo days from 60 to 120 minutes ($P < 0.05$); a maximum between-day difference of 10 percentage points (95% CI, 5–15 percentage points; $P < 0.001$) was observed at 120 minutes (**Figure 4b**).

Corrected QT interval
Corrected QT intervals did not differ statistically significantly at any timepoint (60 and 120 minutes) between days with ILE and corresponding placebo days (differences, −6.0 to 1.2 milliseconds; 95% CI, −15 to 10.8 milliseconds; $P=0.35–0.98$) (data not shown).

Metoprolol plasma concentrations
Plasma metoprolol peak plasma concentration (C_{max})—measured at time to peak plasma concentration = 30 minutes (end of infusion) on both days—was similar on days with (772 nM (206 ng/mL)) and without ILE (830 nM (220 ng/mL)) (mean difference, 58 nM (15 ng/mL); 95% CI, −90 to 205 nM (−24 to 55 ng/mL); $P = 0.94$)) (**Figure 5a,b**). Plasma metoprolol AUCs were also similar (absolute average difference, −8.3 µM × minutes; 95% CI, −60.2 to 43.7 µM × minutes; $P = 0.78$) (**Figure 5c**). Population-level PK fits of plasma metoprolol over time in the presence vs. absence of ILE revealed no effect of ILE on any metoprolol PK parameter (volume of distribution, clearance, and elimination rate constant) (**Methods S1**, PK–PD).

Adverse effects
Adverse effects were reported on 9 days with ILE compared with 0 on days with ILE-placebo. There were no major adverse events, but hematomas in relation to removal of the arterial line >4 cm in circumference were observed by blinded trial personnel on 4 trial days with ILE compared with 0 on days without ILE (**Table S1**, Adverse Effects). In one participant on 1 trial day, a reduction in heart rate to <30 bpm and a stinging sensation in the arm in which infusions were given led to discontinuation of metoprolol and ILE infusions at 18 and 20 minutes, respectively (removing data from this participant had no effects on analyses of metoprolol plasma concentrations). The ILE led to statistically significantly increased triglyceride levels of ≈14 mmol/L (95% CI, 13.5–14.6 mmol/L) on average. Pancreatic amylase levels were numerically but statistically insignificantly increased by on average 2.5 U/L (95% CI, −2.1 to 6.2 U/L) (9–13% from baseline).[20]

DISCUSSION
We investigated whether an ILE dose like that recommended for cardiovascular collapse caused by poisoning with local anesthetics affected hemodynamic parameters with or without a concomitant metoprolol infusion. Furthermore, effects of ILE on plasma metoprolol concentrations and adverse events were investigated.

Intravenous lipid emulsion induced increases in heart rate (up to 5 bpm) and cardiac output (up to 10 percentage points) on ILE days, 1 hour after the end of ILE administration to the 120-minute
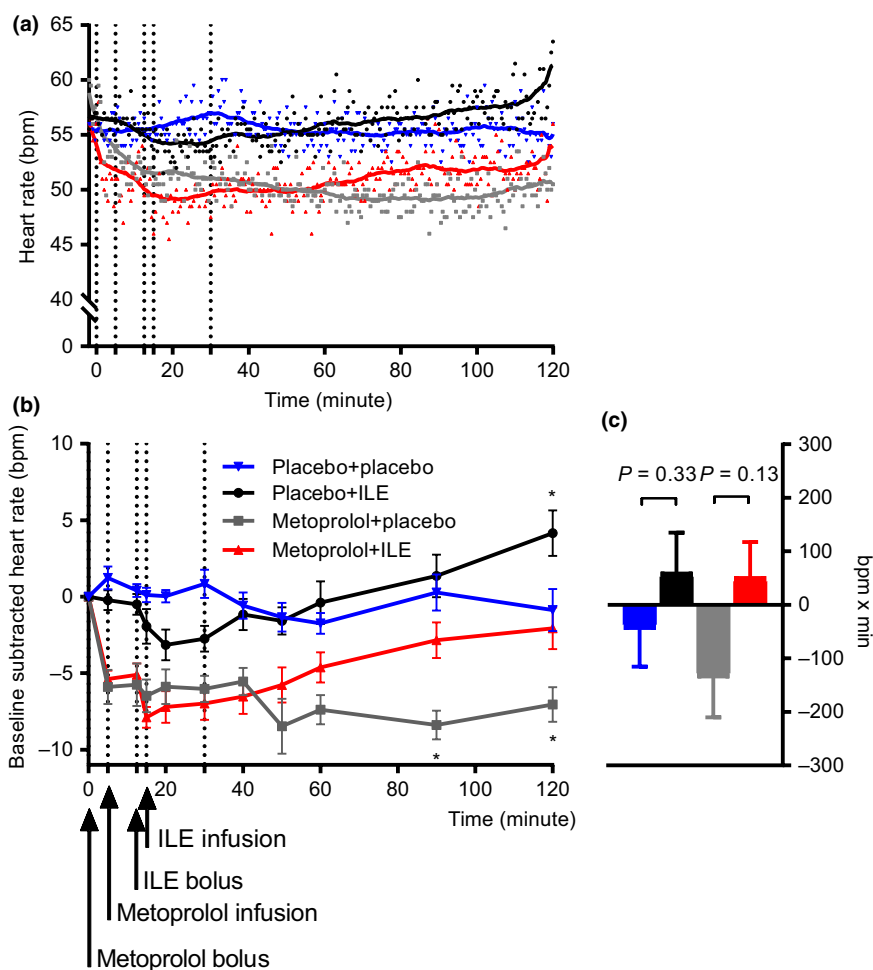
Figure 2 (a) Scatter plot of median heart rates recorded every 30 seconds each day, with locally weighted scatter plot smoothed over 20 adjacent points (LOWESS curve) (blue, placebo + placebo; black, placebo + ILE; gray, metoprolol + placebo; red, metoprolol + ILE). (b) Baseline subtracted heart rates (means ± SEM for each timepoint). Vertical dotted lines and arrows designate start of metoprolol/placebo bolus (0 minutes) and infusion (5 minutes), ILE/placebo bolus (time 12.5 minutes), ILE/placebo infusion (time 15 minutes), and stop of all infusions (time 30 minutes). ILE, intravenous lipid emulsion. *Statistically significant differences between days with ILE compared with corresponding placebo days. (c) Incremental area under the heart rate vs. time curve (mean ± SEM) from ILE/placebo bolus start (time, 12.5 minutes). bpm, beats per minute

timepoint. In the final PK–PD model for heart rate, an initial heart rate decrease due to ILE was predicted. This, together with the linear increase in heart rate in the model further consolidates our findings of a marginal, time-delayed effect of ILE on heart rate (**Methods S1**, PK–PD). Interestingly, the model revealed a limited decrease (~3%) in maximal inhibitory effect of metoprolol, which seems of negligible clinical relevance. A chronotropic effect, together with an increase in cardiac output, may have some clinical relevance. However, the delayed onset of effects would be a clear disadvantage in a clinical situation with acute hemodynamic instability (e.g., cardiogenic shock), in which more rapidly acting therapies would be more favorable. Interestingly, we observed an apparent immediate *decrease* in estimated relative stroke volume and cardiac output during ILE bolus. This may further impede the clinical applicability of ILE in a short-term situation and may even be harmful to a hemodynamically unstable patient.

Interestingly, our findings of modest, gradual effects appear in contrast to the rapid effects (i.e., within minutes) often reported in case reports,[12,21] in which other modalities (e.g., fluids and inotropes) were always used before and alongside ILE and may likely have contributed to the beneficial effects attributed to ILE. The primary proposed mechanism behind rapid effects of ILE insert[22] (i.e., the reversal of toxicity through binding of the poison intravascularly in a lipid compartment) is therefore not likely to play any role in our study. This is perhaps unsurprising: metoprolol has an octanol-water partition add: distribution coefficient (LogD) of −0.34 at physiological pH, we administered ILE corresponding to ≈10–15% of the plasma volume, and we did not observe any effects of the ILE on plasma metoprolol concentrations or metoprolol PK parameters in the population-level PK model. Nonetheless, previous volunteer studies using more lipophilic (e.g., lidocaine[23] and bupivacaine[24]) agents also found no solid evidence of entrapment in the lipid compartment (i.e., a "lipid sink" effect).

Our results agree with previous clinical trials in healthy volunteers, which have found that an ILE + heparin infusion increases heart rate and blood pressure.[25,26] These effects have been
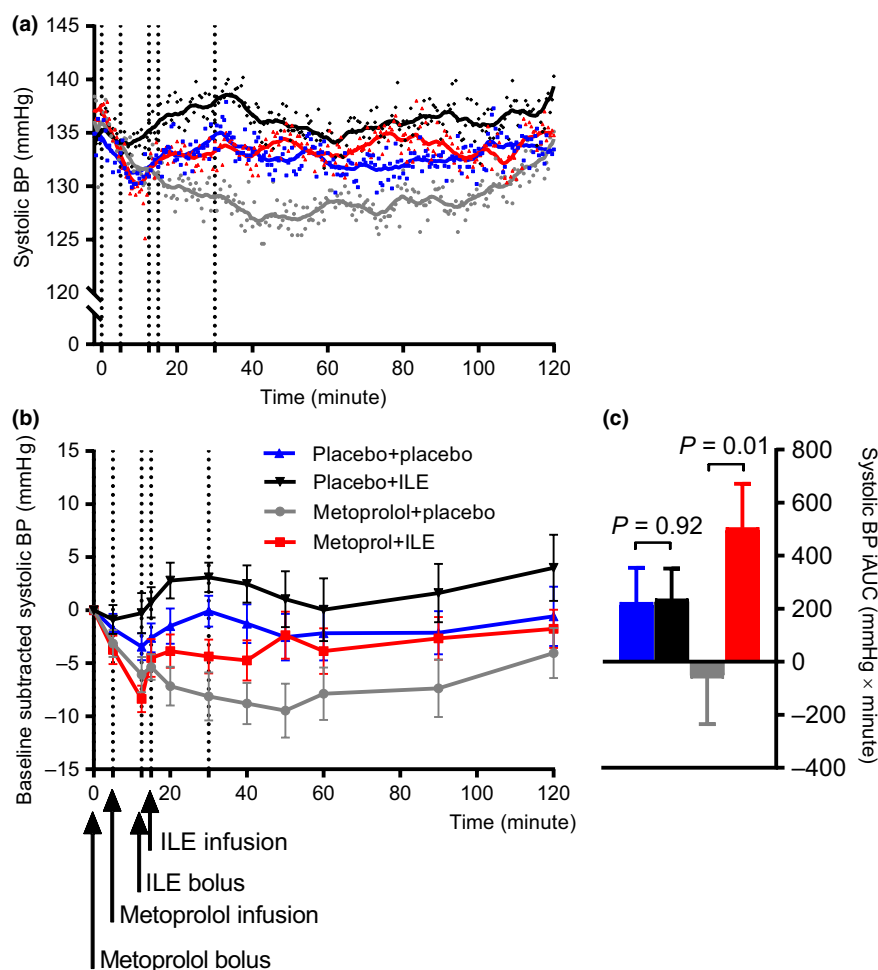
Figure 3 (a) Scatter plot of median systolic blood pressures recorded every 30 seconds each day, with locally weighted scatter plot smoothed over 20 adjacent points (LOWESS curve) (blue, placebo + placebo; black, placebo + ILE; gray, metoprolol + placebo; red, metoprolol + ILE). (b) Baseline subtracted systolic blood pressures (mean ± SEM for each specified timepoint). Vertical dotted lines and arrows designate start of metoprolol/placebo bolus (0 minutes) and infusion (5 minutes), ILE/placebo bolus (time, 12.5 minutes), ILE/placebo infusion (time, 15 minutes), and stop of all infusions (time, 30 minutes). (c) Incremental area under the systolic blood pressure vs. time curve (mean ± SEM) from ILE/placebo bolus start (time, 12.5 minutes). No statistically significant differences between days with ILE compared with corresponding placebo days were found. iAUC, incremental area under the curve; ILE, intravenous lipid emulsion.

suggested to be associated with ILE-induced increased catecholamine levels (epinephrine and norepinephrine) apparent 1 hour after an ILE and heparin coinfusion,[25] but the chronotropic effect of ILE also on days with β_1-adrenoceptor blockade in this study may suggest involvement of mechanisms other than endogenous catecholamines (effects of which would be blocked in β-blocker poisoning). ILE-induced vasoconstriction—related to increased vascular α-adrenergic sensitivity[27–29]—may also contribute to the minor increases in diastolic blood pressure and MAP during ILE infusion. We also observed a decrease in average diastolic blood pressure and MAP from baseline to 12.5 minutes on the placebo + placebo days, which complicates interpretation of these end points. Finally and importantly, stimulatory effects on the heart secondary to caloric effects of the large supply of fatty acids[30] may have contributed to the hemodynamic findings. It has been suggested that the hemodynamic stabilizing effect of ILE in poisonings could be attributable to metabolic effects on the heart,[31] but this hypothesis has yet to be confirmed.

The ILE caused no severe adverse effects, in line with previous volunteer studies. We observed hematomas in relation to removal of the arterial line on 4 days with ILE infusions compared with 0 days without ILE (**Table S1**, Adverse Effects). This warrants further investigation because ILE has been reported to induce platelet dysfunction,[32] which could possibly lead to detrimental coagulopathy.

Limitations
We used an intravenous dose of metoprolol only three to six times above the normally recommended dose to ensure safety of the participants. Average C_{max} obtained (≈800 nM or 210 ng/mL) was thus in the high therapeutic to slight supratherapeutic range and much lower than reported fatal mean plasma concentrations of 60 mg/L (range, 4.7–142 mg/L).[33] None of the trial participants developed severe hypotension or bradycardia. Therefore, our experimental setup has limited translation to the complex clinical situation with severe poisoning and cardiodepression, in
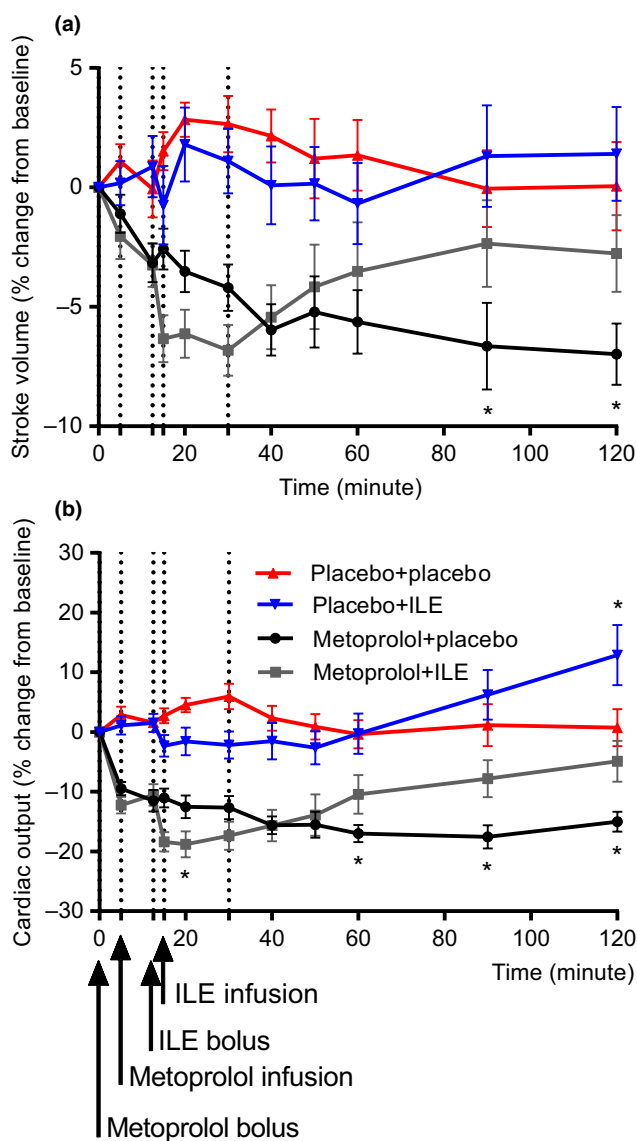
Figure 4 (**a**) Changes in relative stroke volume (percentage changes from baseline, mean ± SEM for each timepoint) (blue, placebo + placebo; black, placebo + ILE; gray, metoprolol + placebo; red, metoprolol + ILE). (**b**) Changes in relative cardiac output (percentage changes from baseline, mean ± SEM for each timepoint). Vertical dotted lines and arrows designate start of metoprolol/placebo bolus (0 minutes) and infusion (5 minutes), ILE/placebo bolus (time, 12.5 minutes), ILE/placebo infusion (time, 15 minutes), and stop of all infusions (time, 30 minutes). ILE, intravenous lipid emulsion. *Statistically significant differences between days with ILE compared with corresponding placebo days.

which many simultaneous interventions (e.g., fluid, atropine, vasopressor therapy, and possibly insulin and glucagon therapy) are typically ongoing. Furthermore, the healthy heart primarily uses fatty acids as energy substrate and may benefit more from ILE compared with the diseased heart in which cellular metabolism is more complicated.[34] The intravenous route of metoprolol administration was chosen instead of the preferred oral route to ensure blinding and limit interindividual and intraindividual variance in

gastrointestinal absorption. Large interindividual variation in metoprolol plasma concentration would, given the few participants in this study, complicate between-day comparisons. This study was not designed for PK–PD analyses, and we performed these post hoc. Important limitations in the PK–PD modeling include too-scarce sampling (i.e., no measurements of serum lipids after 1 hour and clinical values after 2 hours at lower metoprolol and ILE values) and incomplete estimation of volume effects (expansion of plasma volume) due to interventions and placebos.

Strengths
The strength of this study is the randomized, placebo-controlled, crossover design and invasive measurements of hemodynamic end points. Therefore, our study provides reliable data on hemodynamic effects of a large dose of ILE in humans with and without a supratherapeutic dose of the β-blocker metoprolol and adds knowledge concerning the safety of ILE. Dosing schedule was chosen to simulate a situation in which we achieved (small) cardioinhibitory effects of metoprolol *before* ILE administration and in which absorption was ongoing (as is typically the case with overdoses of extended-release tablets) at the time of the intervention with ILE. For ethical reasons, controlled studies involving real-life poisoning situations are difficult to perform, and animal data are not easily generalizable to humans. This underlines the relevance of a clinical trial.

Conclusion
In this double-blinded, randomized, placebo-controlled, crossover trial, ILE induced increases in heart rate (up to 5 bpm) and relative cardiac output (up to 10 percentage points) compared with placebo on days with metoprolol infusion. Effects developed gradually and were statistically significant from ≈1 hour after end of ILE administration to the 120-minute timepoint. Our trial in healthy volunteers may have limited direct clinical translation to a situation with acute cardiovascular collapse. Nonetheless, our observations contrast the often rapid effects of ILE reported in animal studies and clinical cases and thus question the causality of ILE in the resuscitations published as case reports. We observed an abrupt *decrease* in cardiac output and larger hematomas after ILE infusion, which warrant further investigation. In conclusion, we report statistically significant, and relatively marginal and delayed, hemodynamic effects of ILE that may have limited clinical relevance in the short-term clinical toxicological setting.

METHODS
Design and settings
This was a randomized, double-blind, placebo-controlled, four-armed, crossover trial using a repeated Latin square design that was balanced for first-order carryover. The study took place at a phase I trial unit at Bispebjerg University Hospital. It was conducted according to the principles of the Declaration of Helsinki,[35] approved by the Scientific Ethics Committee of the Capital Region of Denmark (registration number H-6-2014-048) and the Danish Medicines Agency (journal number 2016022766), and monitored externally by the Good Clinical Practice (GCP) unit of the Copenhagen University Hospital. The trial is registered on clinicaltrials.gov (identifier NCT02924454—final registration date October 5, 2016).
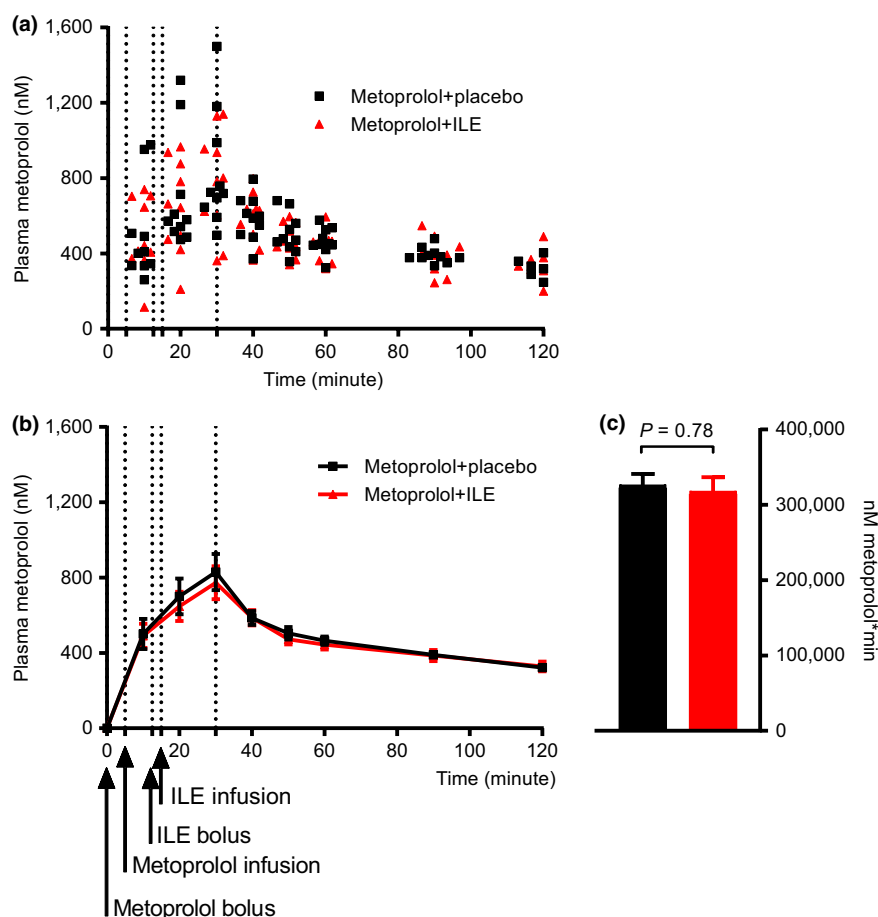
Figure 5 (**a**) All individual plasma metoprolol concentration measures. (**b**) Means ± SEM, on days with coadministration of placebo (black) and ILE (red). Vertical dotted lines and arrows designate start of metoprolol/placebo bolus (0 minutes) and infusion (5 minutes), ILE/placebo bolus (time, 12.5 minutes), ILE/placebo infusion (time, 15 minutes), and stop of all infusions (time, 30 minutes). (**c**) Area under the metoprolol plasma vs. time curve (AUC) on days with placebo (black) compared with days with ILE (red). ILE, intravenous lipid emulsion.

Participants

Eligible participants were men, 20–30 years of age, determined healthy by physical examination, medical history, and laboratory tests (recruitment procedures and trial inclusion and exclusion criteria are shown in **Data S1, Recruitment**).

Randomization and blinding

Before enrollment, a randomization list allocating participants to one of four intervention sequences was generated by personnel otherwise not involved in the trial using an online tool.[36] Participants were included by a blinded investigator according to a randomization list, which was kept in a locked cabinet at the trial site. To avoid unblinding of participants and investigators involved in the assessment of outcome measures, study drugs and placebos were administered behind a curtain by an unblinded research nurse (because ILE is white).

Sample size

Sample size was calculated using the formula: $N = (Z_{2\alpha} + Z_\beta)^2 \times SD^2/$ MIREDIF (minimal relevant difference)^2. $\alpha = 5\%$. Power $(1-\beta) = 80\%$. $Z_{2\alpha}$ and Z_β are the standardized normal deviations corresponding to the selected probabilities. We chose a 10% difference in heart rate as MIREDIF. Metoprolol has been found to reduce heart rate 10% from baseline (SD estimated to be 4%).[37,38] Our chosen MIREDIF would require eight participants. We recruited 10 participants to allow for secondary end points to be explored.

Procedures

Participants underwent the four interventions on separate days (A–D) with a 7-day washout period with combinations of intravenous metoprolol (Seloken; AstraZeneca A/S, Copenhagen, Denmark), ILE (20% Intralipid Fresenius Kabi, Copenhagen, Denmark), or matching placebos (matching volumes of isotonic saline) in randomized order (day A, placebo + ILE; day B, metoprolol + placebo; day C, metoprolol + ILE; day D, placebo + placebo) (**Figure 1**).

Participants were required to be fasting for 10 hours before the trial days. The room temperature was maintained at 21°C. A venous line was inserted into each antecubital vein, and a radial arterial line was inserted in the wrist. Patients rested comfortably in a supine position for a minimum of 15 minutes. Ten milligrams of metoprolol (0.5 mg/mL) or placebo was then administered as a 20-mL bolus injection for 1 minute, followed by a continuous infusion (4 mL/minute from the 5- to the 30-minute timepoint) (the total metoprolol dose was thus 60 mg (120 mL): 10-mg bolus + 50-mg infusion). At 12.5 minutes after the start of the metoprolol/placebo bolus, 20% ILE (200 g/L) or placebo was administered as an initial bolus (1.5 mL/ kg) over 1–2 minutes, followed by continuous infusion (0.25 mL/kg per minute—an infusion rate similar to what is recommended[3]) until the 30-minute timepoint (i.e., a 15-minute infusion period) (**Figure 1**). Bolus and infusions were administered using B Braun Infusomat Space infusion pumps (B Braun AG, Melsungen, Germany). The total ILE/placebo dose (bolus + infusion) was 5.25 mL/kg—corresponding to, on average, 383 mL of ILE/placebo and to 77 g of lipid—≈675 kcal of energy.

Primary outcome measure

The primary outcome measure was changes in heart rate from baseline to 120 minutes (90 minutes after the end of ILE infusion) on days with metoprolol + ILE compared with days with metoprolol + placebo.

Secondary outcome measures

Our secondary outcome measures were effects of metoprolol and ILE on heart rate, estimated relative stroke volume and cardiac output, blood pressure, and corrected QT intervals compared with days with placebos. Furthermore, we analyzed outcomes related to metoprolol PKs (i.e., time to peak plasma concentration, C_{max}, and plasma concentration vs. time AUC of metoprolol on days with coadministration of ILE compared with placebo. This trial involved further exploratory outcome measures (e.g., effects of ILE on laboratory analyses), which are documented on clinicaltrials.gov and have been published elsewhere.[20]

Hemodynamic measurements

Heart rate and arterial blood pressure were recorded every 30 seconds via the radial arterial line connected to an S/3 Datex Ohmeda anesthesia monitor (GE, Helsinki, Finland) from −10 to 120 minutes. Measurements were stored on a computer connected to the anesthesia monitor. Arterial pressure waves were recorded every 10 milliseconds from the anesthesia monitor using custom hardware and software developed in house. Systolic and diastolic pressures were estimated beat to beat from the arterial wave form. Relative stroke volume was calculated using the Liljestrand-Zander pulse-pressure wave equation[39]:

$$SV = k \times \left(\frac{\text{pulse pressure}}{\text{systolic blood pressure} + \text{diastolic blood pressure}} \right),$$

where k is a person-specific constant characterizing the agreement between stroke volume estimated using the equation and measured stroke volume.[40] To remove oscillations of hemodynamic measures caused by, for example, movement of the participant, we used data from the following 2-minute intervals corresponding to timepoints: baseline (minus 2–0 minutes), 5 minutes (4–6 minutes), prelipid bolus (10.5–12.5 minutes), postlipid bolus (14–16 minutes), 20 minutes (19–21 minutes), 30 minutes (29–31 minutes), 40 minutes (39–41 minutes), 50 minutes (49–51 minutes), 60 minutes (59–61 minutes), 90 minutes (89–91 minutes), and 120 minutes (118–120 minutes). To ensure measurement accuracy, the arterial line was zeroed, and atmospheric pressure was set as the reference point. We performed flush tests to ensure damping of the arterial pressure measuring system. Effects on corrected QT intervals (using Fredericia's formula: $QTcF = QT / \sqrt[3]{RR}$) were estimated by single 12-lead electrocardiographic readouts at baseline and 60 and 120 minutes and compared between days.

Plasma metoprolol measurements

Venous blood for measurements of plasma metoprolol was sampled at baseline, 10, 20, 30, 40, 50, 60, 90, and 120 minutes into lithium heparin tubes and centrifuged at 3,000 *rpm* for 4 minutes. Samples were stored in cryotubes at −80°C. Measuring plasma metoprolol was performed as described elsewhere,[41] with minor modifications to handle the sample lipid content. A detailed description of sample handling and analysis is included as **Data S2**, Plasma Metoprolol Measurements.

Statistics

Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC) and GraphPad Prism 7.02 (GraphPad Software, Inc., La Jolla, CA). Hemodynamic end points were analyzed using mixed models of repeated measurements with fixed (intervention, timepoint, and intervention × timepoint) and random (participant randomization number) effects. Tukey's adjustment for multiple comparisons was performed for the hemodynamic end points. Plasma metoprolol concentrations were compared using repeated-measures analysis of variance with Sidak correction. Plasma metoprolol concentration vs. time AUCs and incremental areas from start of ILE/placebo infusion of hemodynamic end points were calculated using the trapezoidal rule and compared between trial days using paired *t*-tests. A two-sided $P < 0.05$ was considered statistically significant. Population-level PK–PD analyses were performed post hoc. PK and PD parameters were analyzed using a nonlinear mixed-effect model with the software package Monolix (Lixoft, Antony, France).[42] Methodological details are presented in **Methods S1**, PK–PD.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Table S1. Adverse effects.
Figure S1. Mean arterial blood pressure.
Figure S2. Diastolic blood pressure.
Methods S1. PK–PD.
Data S1. Eligibility criteria and recruitment procedures.
Data S2. Measurement of plasma metoprolol.

CONFLICTS OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

K.M.P., M.C., S.B., T.B.J., and T.S.P. wrote the manuscript; M.C., K.M.P., T.S.P., S.B., and K.P.D. designed the research; K.M.P., S.B., T.S.P., and M.C. performed the research; K.M.P., M.C., S.B., and T.S.P. analyzed the data; T.H. and H.E.P. contributed new reagents/analytical tools.

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