Effect of Intralipid® on the Dose of Ropivacaine or Levobupivacaine Tolerated by Volunteers

A Clinical and Pharmacokinetic Study

Pauline Dureau, M.D., Beny Charbit, M.D., Ph.D.,† Nathalie Nicolas, M.D., Dan Benhamou, M.D., Jean-Xavier Mazoit, M.D., Ph.D.

ABSTRACT

Background: Rapid intravenous administration of lipid emulsion has become the standard treatment of severe local anesthetic systemic toxicity. This experiment in volunteers aimed at determining the effect of Intralipid® administration on the time to neurologic symptoms.

Methods: Ropivacaine or levobupivacaine was infused intravenously in 16 volunteers (8 mg/min up to 120 mg) with 120 ml Intralipid® 20% (Fresenius, Paris France) or placebo infused at T + 2 min). Each subject received all four treatments in a crossover manner. The infusion was stopped after the intended dose had been administered or on occurrence of incipient neurologic signs of toxicity. The primary outcome was time-to-event. In addition, blood ropivacaine and levobupivacaine concentrations were measured.

Results: The dose infused was not different whether volunteers received placebo (81.7 ± 22.3 *vs.* 80.8 ± 31.7 mg, ropivacaine *vs.* levobupivacaine) or Intralipid® (75.7 ± 29.1 *vs.* 69.4 ± 26.2 mg, ropivacaine *vs.* levobupivacaine), $P = 0.755$, Intralipid® *versus* placebo groups. Plasma concentrations were best modeled with an additional volume of distribution associated with Intralipid®. Simulations suggested that decreased peak concentrations would be seen if Intralipid® was given during a period of increasing concentrations after extravascular administration.

Conclusions: At modestly toxic doses of ropivacaine or levobupivacaine, we were unable to find any effect of the infusion of Intralipid® on the time to early signs of neurologic toxicity in volunteers. Peak concentration was decreased by 26 to 30% in the subjects receiving Intralipid®. Simulations showed that Intralipid® might prevent the rapid increase of local anesthetic concentration after extravascular administration. **(Anesthesiology 2016; 125:474-83)**

CONVULSIONS and/or severe arrhythmias are the main clinical manifestations of severe local anesthetic systemic toxicity.[1] Based on several laboratory studies since 1998[2] and case reports since 2006,[3,4] rapid intravenous administration of lipid emulsion ("lipid rescue") has become the standard treatment of local anesthetic systemic toxicity. The mechanism of action of lipid rescue is still controversial. Several hypotheses have been advanced, among which the "lipid sink" and the metabolic theories are the most popular.[5–7] In the "lipid sink" theory, lipid emulsion is supposed to entrap hydrophobic molecules in the chylomicrons equivalents. We have shown *in vitro* that bupivacaine, and to a lesser extent ropivacaine, binds to the long-chain emulsion Intralipid® (Fresenius, Paris, France) and to a long- and medium-chain emulsion Medialipid® (B. Braun, Boulogne-Billancourt, France).[6] This binding is purely entropic (passive) and follows the classic rules of chemistry. Ropivacaine has a lower partition coefficient than bupivacaine, and Medialipid® particles showed a 40% capacity of binding compared to that of Intralipid®. Animal models of "lipid rescue" show marked discrepancies in their results, likely because of the differences in animal species and protocol designs.[8–11] Interestingly, the partition coefficient of the local anesthetic

What We Already Know about This Topic

- Rapid intravenous administration of lipid emulsion has become a standard treatment of local anesthetic systemic toxicity
- The lipid sink theory postulates that lipid rescue works by entrapping hydrophobic local anesthetic molecules in chylomicron equivalents

What This Article Tells Us That Is New

- In a crossover study conducted in 16 volunteers, a lipid emulsion infusion begun 2 min after initiating an infusion of ropivacaine or levobupivacaine did not affect the times to early signs of central nervous system toxicity
- Peak local anesthetic concentrations at the end of the local anesthetic infusions decreased by 26 to 30% due to an increase in the central volume of a multicompartmental pharmacokinetic model
- Pharmacokinetic simulations suggest that a lipid emulsion might prevent the rapid increase of local anesthetic concentrations after extravascular administration

†Deceased. This article is featured in "This Month in Anesthesiology," page 1A. Corresponding article on page 451. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). Presented in part at the Société Française d'Anesthésie et Réanimation (SFAR) Annual Meeting, Paris, France, September 19, 2014.

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and the concomitant use of epinephrine and lipid seem to markedly influence the results.[12–14] In humans, numerous successful case reports of "lipid rescue" have been published in the last 8 yr,[3,4,15,16] but possible failures have also been published.[17,18] In addition, studies in volunteers failed to show an effect of Intralipid® pretreatment on the onset of neurologic signs of toxicity after lidocaine infusion or on the bupivacaine fraction untrapped in chylomicrons.[19,20] Also, a pharmacokinetic simulation study by Kuo and Akpa[21] suggests that lipid emulsion may not reduce cardiac bupivacaine toxicity by more than 11%.

We designed a study to test the effects of infusion of a lipid emulsion (Intralipid®) given during infusion of a local anesthetic of ropivacaine and levobupivacaine (the two single-enantiomer long-acting agents) on early central nervous system (CNS) and cardiovascular effects in volunteers using a methodology previously used to compare local anesthetic toxicity.[22–25] We combined this clinical study with a pharmacokinetic analysis and simulations to test the validity of the lipid sink theory.

Materials and Methods

After approval by our local ethical committee (Comité pour la Protection des Personnes, Ile-de-France 6, Paris, France) and written informed consent was obtained, 16 healthy adult volunteers (8 males and 8 females) entered the study. Volunteers were judged healthy based on previous medical history, clinical examination, routine blood tests, standard 12-lead electrocardiogram, and electroencephalogram. No subject was receiving any medication or had a history of allergic reaction to local anesthetics, and all were nonsmokers. The study was registered in the ClinicalTrial.gov database with the number NCT01602250, February 23, 2012 (principal investigator: Dr. Benhamou).

Drug Administration

Drugs were administered according to a randomized, double-blind, crossover design (replicated Latin square balanced with respect to carryover effect). Before the study, all subjects were familiarized with the early central nervous toxic effects of intravenous lidocaine. Volunteers received up to 200 mg lidocaine intravenously at a rate of 15 mg/min, and those who did not experience any effects were excluded. After this test session, each responding subject received in a crossover manner, with a washout time greater than 1 week, the following four combinations: levobupivacaine or ropivacaine with Intralipid® or placebo. Randomization was centralized. Volunteers were admitted at 8:00 AM at the Centre d'Investigation Clinique (CIC Paris-Est Hôpital de la Pitié-Salpêtrière Boulevard de l'Hôpital, Paris, France) after an overnight fast on a day-care basis. Two intravenous catheters were introduced in forearm veins (one on each side). Routine monitoring included electrocardiogram, pulse oximetry, and noninvasive arterial pressure. Eight-lead electroencephalogram was continuously monitored before the beginning of infusion and until 60 min after. Twelve-lead electrocardiogram was also continuously recorded at 500 Hz on a Cardioplug digital recorder (Cardionics Inc., Belgium).

Ropivacaine or levobupivacaine was infused at a rate of 8 mg/min (maximum dose 120 mg). Two minutes after the beginning of drug infusion, 120 ml Intralipid® 20% or saline was infused in 1 min on the contralateral forearm. Because it was impossible to obtain a placebo with similar appearance as Intralipid®, blinding was done by masking the entire half of the subject. In addition, the nurse who injected the solution was not involved in any other part of the study. The primary endpoint was the time to onset of CNS toxicity.[22–25] When the subject reported early signs of toxicity, infusion was immediately stopped.

Blood Sampling

Venous blood was sampled on heparinized tubes on the arm opposite to infusion before administration (T0); at the end of local anesthetic infusion (T0′); and 2, 5, 8, 12, 20, 30, 45, 60, 120, 180, 240, 360, and 480 min after T0′. Plasma was separated within 30 min and stored at −80°C until assayed.

Electrocardiographic Recordings

Before administration, at the end of infusion, and 1, 2, 5, and 10 min after infusion, 10-s digital electrocardiograms were sampled from the continuous digital recording device. All electrocardiograms were read in a random order by the same blinded investigator. QRS duration and PR and QT intervals were measured manually directly on the computer screen by changing the position of cursors indicating the start and the end of cardiac intervals following the Common Standards for quantitative Electrocardiography guidelines.[26] QRS duration was defined as the average of three consecutive QRS values. Baseline QRS, PR, and QT were assessed as the mean of three electrocardiogram recordings obtained within 15 min before drug administration. QT was corrected (QTc) using the Fridericia correction. In addition, heart rate and noninvasive arterial pressure were recorded every 2 min.

Drug Assay

Ropivacaine and levobupivacaine were measured using gas chromatography, with a limit of quantification at less than 0.01 mg/l for the two drugs (Supplemental Digital Content, http://links.lww.com/ALN/B297).[27,28] The intra- and inter-day coefficients of variation were 6 and 8% at 0.2 mg/l in the absence of Intralipid® and between 10 and 16% in the presence of Intralipid®, depending on the emulsion concentration. In the absence of Intralipid®, the fraction extracted (recovery) is 97 to 102% in plasma. However, because Intralipid® may have decreased the efficacy of extraction, we also measured ropivacaine and levobupivacaine (0.5 and 4 mg/l) *in vitro* in plasma in the presence of various Intralipid® dilutions (1/10, 1/25, and 1/100). Three conditions of extraction were tested: (1) immediate assay after mixing, (2) after rapid centrifugation at 20,800*g* for 10 min,[20] and (3) rapid freeze

of the mixed solution at −80°C and assay of the thawed samples. It was not possible to adequately measure the free drug concentration in the presence of Intralipid® likely because of polarization of the separating membranes.[6] Accordingly, we report only the total drug concentration (free drug in plasma water + drug in plasma proteins + drug in the lipid moiety). Plasma lipase was measured at T + 2 h in the sequences with Intralipid® using a standard automated assay.

Pharmacokinetics

A nonlinear mixed effect (population) analysis was performed using NONMEM VI (NONlinear Mixed Effects Model)[29] considering the principle of parsimony (Supplemental Digital Content, http://links.lww.com/ALN/B297). The total drug concentration–time data were best described by an open two-compartment linear model. An additional volume of distribution related to Intralipid® was added to the central compartment. The following parameters were estimated: total body clearance (CL); intercompartmental clearance between compartments 1 and 2 (Q2); volume of the central compartment (V_1); volume of the peripheral compartment (V_2); volume of the Intralipid® compartment (V_{IL}); terminal half-life ($T_{1/2}$); and lag time (T_{lag}). The maximum observed concentration (C_{max}) is also reported. The precision of estimation of structural (fixed) parameters was calculated using log-likelihood profiling. Because of the complex structure of the data (multiple nesting), we did not bootstrap the data, and the interindividual variability is reported as ω^2, the variance of the random parameters associated with fixed parameters. Models were compared using the log-likelihood ratio test or the Akaike criterion depending on parameter nesting. Because of the asymptotic nature of fitting, we considered $P = 0.01$ as the limit of significance in the pharmacokinetic part. Normalized prediction distribution errors (NPDE) were calculated to assess goodness of fit.[30]

In addition, we performed simulations using the estimated parameters. These simulations aimed at visualizing the effect of a 120-ml bolus of Intralipid® 20% injected after extravascular route of administration of moderately high doses of ropivacaine and levobupivacaine.

A noncompartmental analysis was also performed. The terminal half-life ($T_{1/2}$) was calculated using log linear regression of the observed terminal curve, the area under the curve was calculated using the trapezoidal rule and extrapolated to infinity, and the total body clearance (CL) was calculated as D/area under the curve, where D is the dose injected.[31] $T_{1/2}$ and CL were compared using the procedure linear mixed effect (lme) of R.[32,33]

Statistical Analysis

Assuming that the mean time until appearance of toxicity in subjects receiving either ropivacaine or levobupivacaine and placebo will be 3.8 ± 1.2 min,[23] we considered that 16 subjects receiving the four treatments were required to detect a difference of 45 or 35% with a residual error variance of 0.5 or 1.0, respectively, and α and β errors of 0.05 and 0.1, respectively (using contrasts). Sex stratification randomization was performed. The normality of data was checked when required using Q–Q plots and the Shapiro test. Time-to-early signs, heart rate, arterial pressure, QRS duration, and PR and QT intervals were analyzed with the procedures ezANOVA or lme when data were missing, with drug and Intralipid®/placebo as fixed effects (between factors), the difference from baseline as within factor, sex as covariate, and subjects as random effects. Carryover effect was initially searched by testing the effect of sequences. C_{max}, the observed maximum concentration, was analyzed with the procedure lme with subject as random effect. For better representation of the concentration–time curves, we added smoothed curves using the procedure supsmu. Results are expressed as mean ± SD unless otherwise specified. Statistical significance was considered at $P < 0.05$.

Results

Demography and Dose

From January 2012 to August 2013, 25 volunteers were screened. Six were not included because of previous history of generalized seizure, electrocardiogram abnormalities, or spasmophilia. Of the 19 subjects enrolled, one was not included for lack of CNS toxicity symptoms after 200 mg lidocaine and one for severe anxiety. Seventeen were eligible to continue the study after the lidocaine test. One was excluded after the second session for major difficulties of blood sampling. Only the results of the 16 volunteers (8 males and 8 females) who finished the study are reported. Their mean age, height, and body weight were 29 ± 6 yr, 172 ± 8 cm, and 69.9 ± 9.7 kg, respectively. Baseline values of weight, heart rate, arterial pressure, oxygen saturation, and global physical condition did not differ between each phase.

The mean dose of lidocaine administered in the first session was 122 ± 50 mg (range, 49 to 195 mg). The mean total dose (exactly related to time of administration) of the study drug administered is depicted in table 1. Neither carryover nor investigator effect was detected. There was no statistical difference in dose between the ropivacaine/levobupivacaine groups ($P = 0.317$) nor between the placebo/Intralipid® groups ($P = 0.755$). Four subjects received the maximum dose (120 mg): one volunteer in the levobupivacaine + placebo and levobupivacaine + Intralipid® sessions and the other three in the levobupivacaine + placebo, levobupivacaine + Intralipid®, and ropivacaine + Intralipid® sessions.

No severe adverse effect occurred. The CNS symptoms reported are listed in table 2. The most common symptoms were dizziness and dysarthria. CNS symptoms disappeared within 5 min after the end of infusion.

Table 1. Dose of Ropivacaine or of Levobupivacaine Received by the Volunteers (Note that Doses and Duration of Administration Are Totally Correlated)

	Session			
	Ropivacaine + Placebo	Ropivacaine + Intralipid®	Levobupivacaine + Placebo	Levobupivacaine + Intralipid®
Tolerated dose				
mg	81.7 ± 22.3	75.7 ± 29.1	80.8 ± 31.7	69.4 ± 26.2
mg/kg	1.18 ± 0.40	1.14 ± 0.50	1.18 ± 0.49	1.00 ± 0.36
Duration of infusion (min)	10.21 ± 2.8	9.46 ± 3.6	10.11 ± 3.97	8.67 ± 3.27

There is no statistical difference between the ropivacaine/levobupivacaine groups ($P = 0.317$) nor between the placebo/Intralipid® groups ($P = 0.755$).

Electrocardiographic and Electroencephalographic Findings

Heart rate and systolic blood pressure significantly increased after lidocaine infusion from 69 ± 10 to 77 ± 12 bpm, $P = 0.0025$, and from 117 ± 9 to 123 ± 8 mmHg, $P = 0.011$, respectively. Heart rate and systolic blood pressure significantly increased after levobupivacaine or ropivacaine administration from 69 ± 10 to 73 ± 8 bpm, $P = 0.014$, and from 119 ± 13 to 128 ± 10 mmHg, respectively. Neither type of drug nor Intralipid®/placebo had a significant effect on these changes. QRS duration, PR, and QTc intervals at baseline and their change with time are reported in table 3. Both ropivacaine and levobupivacaine were associated with a significant increase in QRS duration (approximately 5 ms, $P < 0.0001$) and QTc (12 to 20 ms, $P < 0.0001$) with time but without any effect of Intralipid® and no difference between the two local anesthetics. No significant increase in PR interval was observed. No electroencephalographic change was recorded.

Drug Assay and Pharmacokinetics

Nine hundred and twenty-nine samples were analyzed (31 samples were missing due to sampling difficulties). The presence of Intralipid® in plasma altered the extraction efficacy, possibly leading to incomplete recovery (supplemental table, Supplemental Digital Content, http://links.lww.com/ALN/B297). However, after freezing and thawing, full recovery was obtained with our modified extraction procedure. The concentration measured was then the total drug in plasma, *i.e.*, the free drug in plasma water + the drug bound to proteins + the drug entrapped in the Intralipid® droplets or bound to the free fatty acids, glycerol, or lecithins. As we noticed earlier, it was not possible to adequately measure the free drug concentration in the presence of Intralipid® likely because of polarization of the separating membranes.[6]

The observed peak concentration (C_{max}) was significantly lower in the levobupivacaine–placebo than in the ropivacaine–placebo group (table 4). The Intralipid® infusion significantly reduced C_{max} for both local anesthetic treatment groups compared to their saline controls ($P = 0.015$) (figs. 1 and 2).

Different two- and three-compartment open models were tested (Supplemental Digital Content, http://links.lww.com/ALN/B297). In order to model the effect

Table 2. Early Signs of Central Nervous System Toxicity Reported by the Subjects in the 16 × 4 Sessions (More than One Symptom Is Usually Reported)

Symptoms	N (%)
Dysarthria	45 (56)
Dizziness	44 (55)
Tinnitus	16 (20)
Circumoral paresthesia	15 (19)
Paresthesia	14 (17.5)
Blurred vision	12 (15)
Tongue numbness	8 (10)
Metallic taste	8 (10)
Myoclonia	2 (2.5)
Hearing disturbances	1 (1)

Table 3. Changes in QRS Duration, QT (Corrected), and PR Intervals

	QRS Base (ms)	Delta T0′ (ms)	Delta Max (ms)
Ropivacaine/placebo	92 ± 6	4 ± 4	5 ± 3
Ropivacaine/Intralipid®	91 ± 6	4 ± 4	5 ± 3
Levobupivacaine/placebo	93 ± 6	4 ± 3	5 ± 3
Levobupivacaine/Intralipid®	93 ± 6	3 ± 3	5 ± 3
	QTc Base (ms)*	**Delta T0′ (ms)**	**Delta Max (ms)**
Ropivacaine/placebo	396 ± 21	14 ± 16	20 ± 21
Ropivacaine/Intralipid®	398 ± 20	13 ± 26	18 ± 11
Levobupivacaine/placebo	399 ± 22	12 ± 21	16 ± 19
Levobupivacaine/Intralipid®	399 ± 15	3 ± 14	12 ± 16
	PR Base (ms)	**Delta T0′ (ms)**	**Delta Max (ms)**
Ropivacaine/placebo	161 ± 19	5 ± 9	10 ± 8
Ropivacaine/Intralipid®	161 ± 17	3 ± 6	8 ± 5
Levobupivacaine/placebo	155 ± 24	5 ± 9	8 ± 8
Levobupivacaine/Intralipid®	162 ± 19	5 ± 9	10 ± 12

QRS and QTc significantly increased ($P < 0.0001$) with administration time, but no difference was observed between the placebo/Intralipid® groups, nor between the ropivacaine/levobupivacaine groups. PR interval did not increase significantly. Delta Max is the maximum observed increase in QRS, QT, and PR duration. Delta T0′ is the difference between end of infusion (T0′) and baseline.

*QT was corrected by the Fridericia correction (QTc).

Table 4. Pharmacokinetic Parameters Calculated with NONMEM

	Ropivacaine	Levobupivacaine
C_{max} (mg/l)* (mean ± SD)		
Placebo	1.67 ± 0.82	1.24 ± 0.70
Intralipid®	1.24 ± 0.63	0.87 ± 0.36
CL (l/min)	0.568 (0.478–0.679)	0.686 (0.594–0.812)
	ω^2 = 0.083	ω^2 = 0.102
V_1 (l)	52.4 (44.4–58.8)	67.8 (54.6–84.0)
	ω^2 = 0.113	ω^2 = 2.63
V_{IL} (l)†	5.71 (1.59–16.0)	12.2 (2.49–25.3)
	ω^2 = 5.71	ω^2 = 2.63
Q (l/min)	0.442 (0.294–0.657)	0.708 (0.562-0.97.5)
	ω^2 = 0.442	ω^2 = 0.223
V_2 (l)	47.5 (36.3–64.1)	78.2 (60.5–107)
	ω^2 = 0.107	ω^2 = 0.160
$T_{1/2}$ (min)‡	168/173	197/206
T_{lag} (min)	1.76	

Data are population estimates with their 95% CI obtained from log likelihood profiling. $T_{1/2}$ (placebo/Intralipid®) is the hybrid terminal half-life calculated from individual parameters.

*C_{max} was significantly different between the levobupivacaine–placebo and the ropivacaine–placebo groups (P = 0.015) and within each drug group, between the Intralipid® and placebo groups (P = 0.015). †V_{IL} is for 120 ml Intralipid® 20%. ‡$T_{1/2}$ has no CI because the value was calculated from the other estimates.

CL = total body clearance; C_{max} = observed maximum concentration; NONMEM = NONlinear Mixed Effects Model; Q = intercompartmental clearance from compartments 1 to 2; $T_{1/2}$ = terminal half-life; T_{lag} = lag-time; V_1 = volume of the central compartment; V_2 = volume of peripheral compartment; V_{IL} = additional volume associated with V_1 in the two groups receiving Intralipid®; ω^2 = variance of the interindividual variability parameter associated with the structural parameter.

of Intralipid®, we considered a compartment of distribution (V_{IL}) added to the central compartment. This model is clearly not perfectly adequate (fig. 2), but more sophisticated models led to overparameterization, likely because sampling begun after infusion cessation. A two-compartment open model best described the total concentration–time data (Supplemental Digital Content, http://links.lww.com/ALN/B297). Greater volumes of distribution and greater clearance in the levobupivacaine compared to the ropivacaine groups explained the lower C_{max} in the levobupivacaine group (P = 0.015; fig. 1; table 4). The Intralipid® bolus appeared to increase the volume of the central compartment compared to that of each saline controls by 11 and 18% for ropivacaine and levobupivacaine, respectively. We observed a lag time, which is obvious in figure 1. Venous sampling may possibly explain this phenomenon. All other pharmacokinetic parameters are listed in table 4. The nonparametric analysis gave similar results, with CL only different between drug groups and $T_{1/2}$ not different between the four groups (table 5).

Simulations performed with the estimates of NONMEM pharmacokinetic parameters predicted a noticeable reduction of C_{max} only when the local anesthetic drug concentration is high because the decrease is proportional to concentration in the range of concentrations below saturation (fig. 1). Early after Intralipid® injection, drug distributes in the volume of the central compartment with the ratio $(V_{IL} + V_1)/V_1$. The higher the local anesthetic concentration, the more the effect

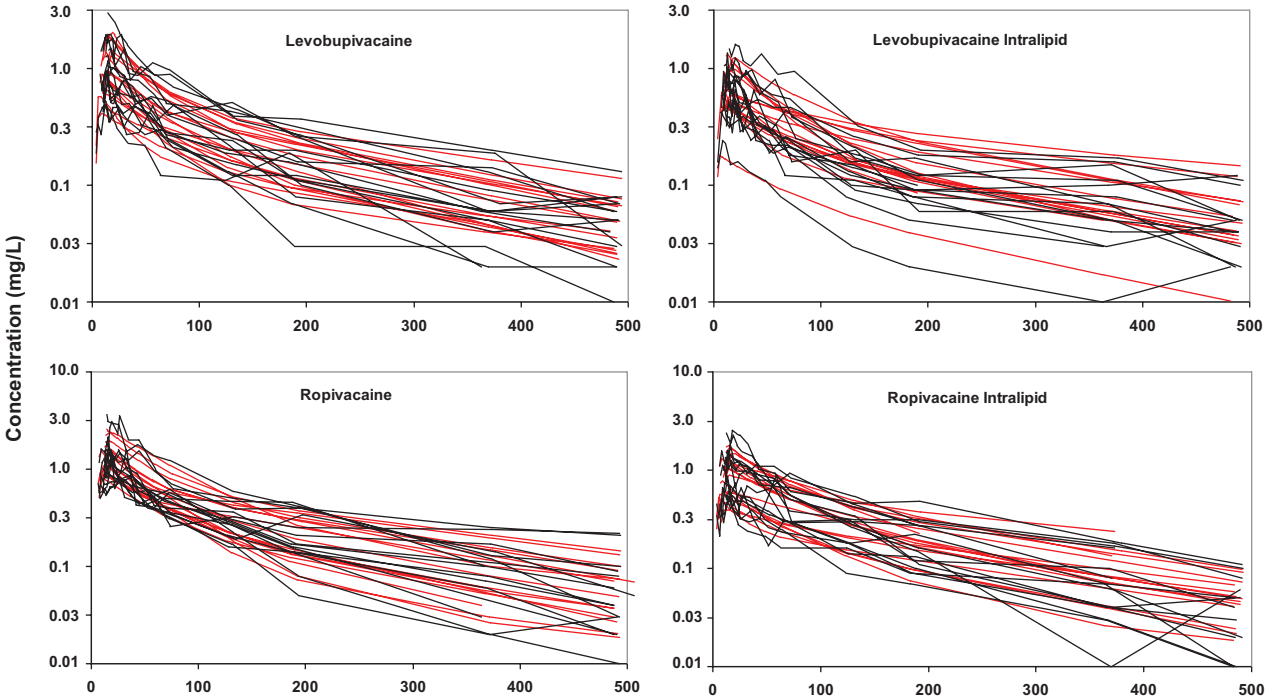

Fig. 1. Concentration–time profile of levobupivacaine and of ropivacaine on a logarithmic scale (base 10). *Black lines* are raw data, *red lines* are the corresponding *post hoc* Bayesian values obtained by smoothing concentration–time data. Clearly, Intralipid® decreased peak concentration in the levobupivacaine group and in a lesser extent in the ropivacaine group. A prolonged effect is not obvious on this representation.

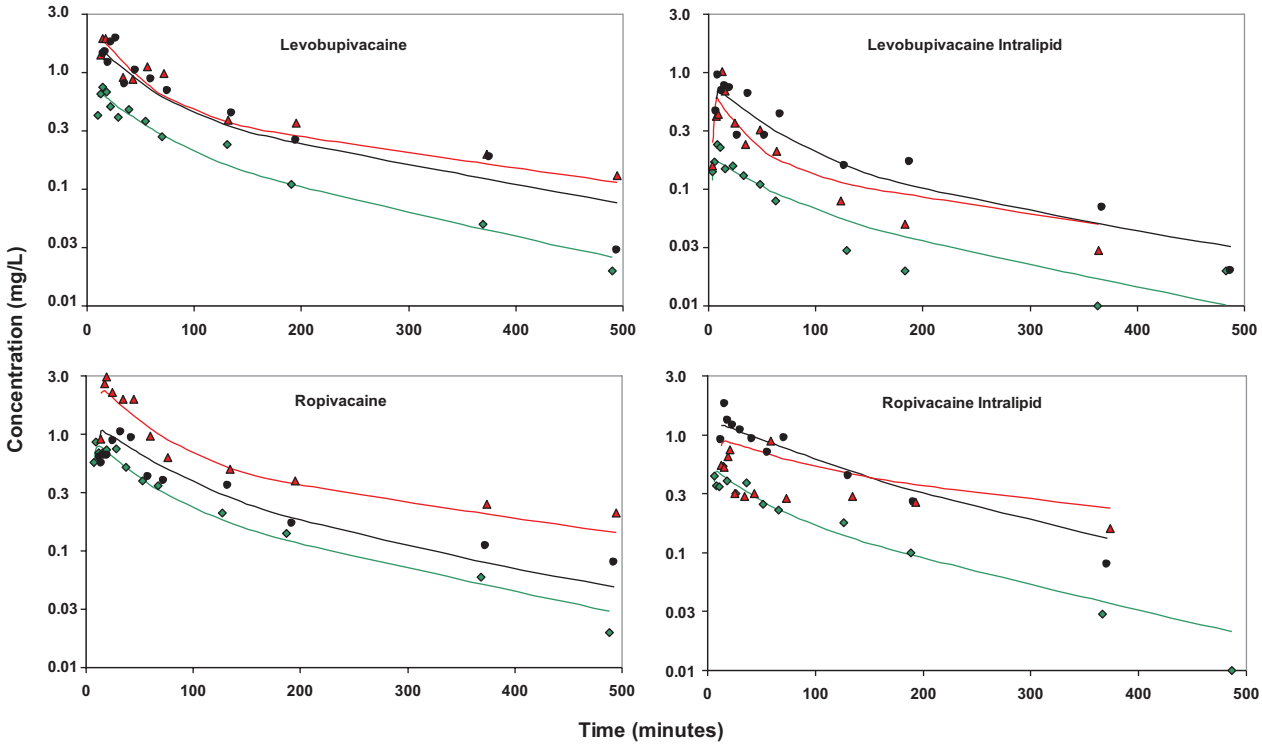

Fig. 2. Best (*green*), median (*black*), and worst (*red*) model fits according to NONMEM IWRES (NONlinear Mixed Effects Model Individual Weighted RESiduals). Shrinkage is important (as in fig. 1). Interestingly, despite the combined proportional and additive error statistical model, best fits correspond to the low concentration sets.

of the lipid emulsion will be observed. Clearly, from these simulations, Intralipid® is likely more efficacious if injected early, *i.e.*, when the local anesthetic concentration in plasma is rising, whereas the effect on an established local anesthetic concentration seems to be less. Plasma lipase concentration was not increased after Intralipid® infusion (30.4 ± 9.2 and 26.9 ± 9.1 U at T0 and T + 2 h, respectively).

Discussion

Intralipid® treatment did not change the time to occurrence of early signs of toxicity seen with infusion of either ropivacaine or levobupivacaine. Dosing was similar, whether or not the subjects received Intralipid®. However, an effect on the pharmacokinetic profile was observed (fig. 1; table 4). This effect, leading to a moderate but significant decrease in C_{max} may possibly explain why "lipid rescue" has shown most probable efficacy in cases of massive intoxication with very high plasma concentrations.[3,4,15,16] This assertion is supported by simulations showing that at high concentrations, Intralipid® may decrease the local anesthetic drug concentration in plasma (fig. 3). Simulations performed by Kuo and Apka[21] using previously published values showed that the expected benefit of the lipid sink may be of moderate benefit in terms of tissue distribution of bupivacaine. Other mechanisms (not mutually exclusive) have been proposed to explain the effect of lipid emulsions on cardiac function.[7,8,34,35] In addition to their effects on myocardial conduction, bupivacaine and ropivacaine markedly decrease contractility, at least partly by interfering with calcium regulation.[36,37] In that respect, it has been recently shown that lipids induce a rapid cardiotonic effect in intact rats and in isolated heart preparation.[34]

Table 5. Nonparametric Analysis

	Ropivacaine		Levobupivacaine	
	Placebo	Intralipid®	Placebo	Intralipid®
CL (l/min)*	0.560 (0.192)	0.617 (0.160)	0.703 (0.296)	0.765 (0.335)
$T_{1/2}$ (min)†	202 (183)	142 (55.8)	172 (80.6)	227 (102)

Clearance was significantly different between ropivacaine and levobupivacaine groups, but not between Intralipid® and placebo groups. $T_{1/2}$ was not statistically different between groups. Data are represented as mean (SD).

*P = 0.0044, ropivacaine *versus* levobupivacaine, P = 0.23 Intralipid® *versus* Placebo. †P = 0.21, ropivacaine *versus* levobupivacaine, P = 0.37 Intralipid® versus Placebo.

CL = clearance, $T_{1/2}$ = terminal half-life.

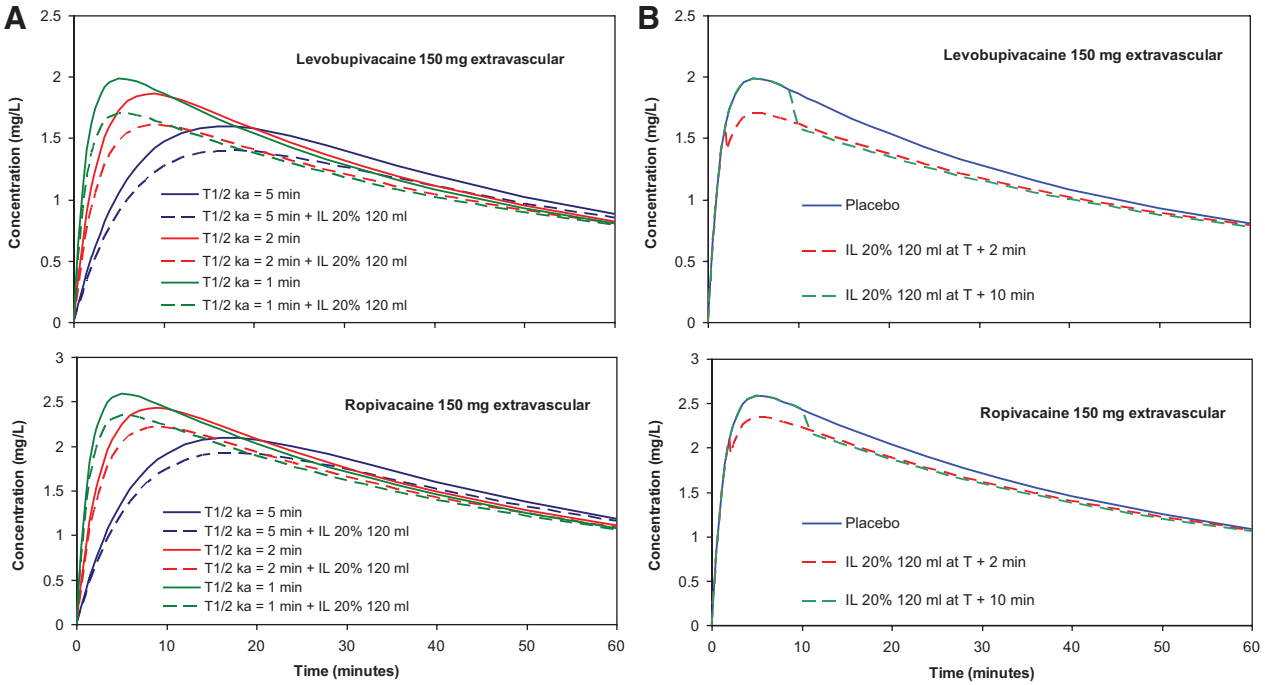

Fig. 3. Simulations of the plasma concentration observed after extravascular injection of 150 mg levobupivacaine or ropivacaine followed by a bolus of 120 ml Intralipid® (IL) 20%. (*A*) The effect of the speed of absorption (Ka, the rate constant of absorption, varying from 0.14 to 0.69 min^{-1}; IL is injected at time = 0). *Plain lines* are placebo groups. *Dotted lines* are IL groups. The decrease of local anesthetic concentration induced by IL is greater in the levobupivacaine than in the ropivacaine group and when absorption is rapid. (*B*) The effect of the time of IL administration (same parameters as in *A*). Although IL administered 2 or 10 min after local anesthetic injection identically puts concentration on almost the same lower curve, it is clear that an early administration of IL (120 ml 20%), *i.e.*, before the onset of peak concentration, may prevent very high concentration leading to toxicity. Please note the difference in the concentration scale between the two drugs. T = time of drug injection; $T_{1/2}$ ka = absorption half-life.

We studied the effects of the two S enantiomers, ropivacaine and levobupivacaine, because these two drugs have proved less toxicity *in vitro* and *in vivo*.[21–23,38] We designed the present protocol assuming a 35 to 45% increase in the dose of the local anesthetic infused in the lipid treatment group. The lipid emulsion infusion was performed 2 min after the initiation of local anesthetic infusion in order to be as close as possible to the clinical situation, but without the possibility of appearance of signs of toxicity in a volunteer before the lipid infusion began. However, no effect of Intralipid® was seen on the total dose infused (table 1) or on the electroencephalogram recordings. In a study designed to test the effect of a preventive Intralipid® bolus on the occurrence of subjective CNS symptoms and electroencephalogram modifications after intravenous lidocaine administration, Heinonen *et al.*[19] report similar findings. Moreover, we did not find any significant effect of Intralipid® on the electrocardiogram. Ropivacaine and levobupivacaine significantly increased QRS duration and QTc interval in a similar order of magnitude as already described,[22–25] but no significant effect of Intralipid® was observed (table 3). Because of ethical considerations, it was impossible to infuse larger doses of local anesthetics, and it is not possible to draw definite conclusions on what effect lipid infusion might have on highly toxic concentrations.

The presence of Intralipid® in the sampled plasma complicated the assay's performance. Contrary to the findings of Litonius *et al.*[20] we were unable to accurately measure the free drug concentration in the presence of Intralipid® despite similar methods. We already noticed this earlier[6] and concluded that polarization and fouling of the ultrafiltration membrane were likely the cause. In addition, since our usual extraction procedure did not allow us to obtain total recovery in the samples containing Intralipid®, we modified the procedure (Supplemental Digital Content, http://links.lww.com/ALN/B297).

We were unable to fit a three-compartment model to the data, likely because of delayed sampling (Supplemental Digital Content, http://links.lww.com/ALN/B297). However, ropivacaine and levobupivacaine pharmacokinetics were similar to those reported earlier after intravenous administration[39–41] but best described by an open two-compartment model contrary to the previous population analysis of Olofsen *et al.*[41] (table 4). Our estimates of central volumes were higher to those previously reported by authors who performed arterial sampling[41] (table 4). This is in agreement with the delayed sampling, the fact that the drugs rapidly distribute in the periphery and that the arterial and venous concentration–time curves differ until 2 h after extravascular administration.[42] The peak concentration

observed after infusion was stopped (C_{max}) was markedly lower (26 and 30% for ropivacaine and levobupivacaine, respectively) when the volunteers received Intralipid® (fig. 1). When corrected for the dose, this decrease corresponds to an effect size (Cohen d) of 0.51 and 0.56 for ropivacaine and levobupivacaine, respectively. However, total body clearance was not affected by lipid administration (tables 4 and 5), likely because the effect of Intralipid® is expected to be of short duration.[43–45] This lower peak concentration was associated with a marked increase in the central volume of distribution. This volume (V_{IL}), the addition of which significantly improved the model ($P < 0.0001$), was 5.71 and 12.2 L (ropivacaine *vs.* levobupivacaine) for 120 ml Intralipid® 20% infused. The difference in V_{IL} between ropivacaine and levobupivacaine is in the same order of magnitude as in our previous publication[6] and to the respective partition coefficient of the two drugs. Total body clearance was not significantly different between the groups receiving placebo or Intralipid®. We may then speculate that the half-life of disappearance of V_{IL}, the volume added to V_1, was short. Published *in vivo* half-lives of chylomicrons and Intralipid®-triglycerides are approximately 7 and 14 min, respectively.[43,44] However, degradation of Intralipid® chylomicrons releases free fatty acids, glycerol, and lecithins that also bind local anesthetics, but to a lower extent. In addition, liposomes present in the emulsions may participate in the binding process.

Because lipid emulsions displace numerous drugs from their sites of binding on α1 acid–glycoprotein and on the site II of albumin,[46,47] it would have been interesting to test whether displacement of protein-bound drug would have occurred. In addition, any change in free fraction induced by lipid would have changed the volume of distribution by itself. Unfortunately, we were unable to accurately measure the free drug concentration when the subjects received Intralipid®.

Intralipid® injection for rescue has been implicated by some authors in the development of possible adverse reactions including pancreatitis.[48] Plasma lipase concentration in the volunteers did not increase 2 h after administration (a pilot analysis measuring lipase concentration in two volunteers from T0 to T + 8 h revealed a constant low concentration).

Simulations were performed to test the expected effect of lipid emulsion at toxic local anesthetic concentrations (fig. 3). These simulations are only speculations with numerous simplifying assumptions. Importantly, kinetic parameters were considered constant (no effect of cardiovascular impairment was taken in account). Clearly, these simulations show that a bolus injection of Intralipid® shortly after local anesthetic drug administration is able to markedly reduce an increase in concentration, but not to substantially decrease a near-plateau concentration. In a physiologically based simulation study, Kuo and Akpa[21] find a 10 to 20% decrease in CNS and heart tissue bupivacaine concentration after Intralipid® administration (bolus 1.5 ml followed by 0.25 ml kg^{-1} min^{-1} infusion). Thus, despite an important binding capacity of the lipid emulsion, the lipid sink effect does not appear to be of major intensity. An Intralipid® bolus injection seems to be able to reduce high peak concentrations observed after rapid absorption: the higher the anesthetic drug concentration in plasma, the more efficacious would be the lipid emulsion.

In conclusion, at moderately toxic doses of ropivacaine or levobupivacaine administered in volunteers, we were unable to find any effect of the infusion of Intralipid® on the time to early signs of CNS toxicity. Similarly, the infusion of Intralipid® did not modify the observed increase in the QRS duration and the QTc interval. Contrary to these clinical findings, the pharmacokinetics was altered by the infusion of Intralipid®. $C_{max,}$ the peak concentration observed at the end of the local anesthetic infusion, was decreased by 26 to 30% in the subjects receiving Intralipid®, thus supporting at least partly the "lipid sink" hypothesis. Kinetic simulations showed that Intralipid® might prevent the rapid increase in concentration of the local anesthetic drug after extravascular administration.

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Competing Interests

The authors declare no competing interests.

Reproducible Science

Full protocol available from Dr. Mazoit: jean-xavier.mazoit@u-psud.fr. Raw data available from Dr. Mazoit: jean-xavier.mazoit@u-psud.fr.

Correspondence

Address correspondence to Dr. Mazoit: Faculté de Médecine, Laboratoire d'Anesthésie, 63 rue Gabriel Péri 94276 Bicêtre Cedex, Paris, France. jean-xavier.mazoit@u-psud.fr.

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