Lipid-free Fluoropolymer-based Propofol Emulsions and Lipid Reversal of Propofol Anesthesia in Rats

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ABSTRACT

Background: Propofol, as a lipid-based emulsion, is effective at inducing anesthesia. It does, however, suffer from several drawbacks, including microbial growth, hyperlipidemia, and pain on injection. In this study, the authors examined the ability of four lipid-free propofol nanoemulsions to induce anesthesia in rats and tested whether a subsequent lipid bolus would accelerate emergence from anesthesia.

Methods: The authors administered five formulations of propofol intravenously to six rats, delivering five different doses five times each, in a repeated-measures randomized crossover design and measured time to loss and recovery of righting reflex. The formulations included (1) Diprivan (AstraZeneca, United Kingdom); (2) L3, incorporating a semifluorinated surfactant plus egg lecithin; (3) B8, incorporating a semifluorinated surfactant only; (4) F8, incorporating a semifluorinated surfactant plus perfluorooctyl bromide; and (5) L80, incorporating egg lecithin only. In a second phase of the study, the authors administered a lipid bolus immediately after a dose of B8 or Diprivan.

Results: All formulations except L80 impaired the righting reflex without apparent toxic effects. The authors estimated the threshold dose for induction by determining the x-intercept of the linear regression between time to recovery versus log dose. Threshold doses ranged from 5.8 (95% CI, 5.5 to 6.2) to 8.6 (95% CI, 7.2 to 10.2) mg/kg. A 15 ml/kg lipid bolus resulted in an accelerated clearance.

Conclusions: Three of the four novel lipid-free fluoropolymer-based formulations showed efficacy in producing anesthesia, which was comparable to that of Diprivan, and a lipid bolus hastened recovery. These novel propofol formulations have the potential to avoid complications seen with the existing lipid-based formulation. (Anesthesiology 2016; 124:1328-37)

Propofol is commonly used for induction and maintenance of general anesthesia, as well as for sedation in the operating room and intensive care unit. It was initially formulated in Cremophor EL (BASF Corp., Germany), but this excipient produced unacceptable levels of toxicity, including anaphylactoid reaction.1 The formulation currently available (Diprivan; AstraZeneca, United Kingdom, also available in generic versions) is a lipid-based emulsion of 1% propofol in 10% soybean oil, 1.2% egg yolk lecithin, and 2.25% glycerol.2 This formulation is clinically effective, but there remain several drawbacks, including instability,3,4 opportunity for microbial growth,5–7 effects related to hyperlipidemia (elevated triglycerides and propofol infusion syndrome),8–11 and pain on injection.12 Although rare, anaphylaxis has also been of concern.13,14 There have been many attempts to remedy these issues. Preservatives and antimicrobial agents such as EDTA and sodium metabisulfite have been added.1,15 Oil and lecithin content has been varied.16 Different sizes of triglycerides and new solvents have been tested.17,18 High concentrations of free propofol produced by rapid release from the oil phase, acting on transient receptor potential A1 receptors,19 have been implicated in pain on injection.20–22 Therefore, emulsions have been developed to decrease the concentration of free propofol, attempting to minimize this problem.23,24 Prodrugs of propofol (fospropofol) are available and have decreased pain on injection but have slower onset and prolonged elimination half-life.25 Recently, Aquafol (Daewon Pharmaceutical Co., Ltd., Korea), a 1% propofol microemulsion with 10% purified poloxamer 188 and 0.7% polyethylene glycol 660 hydroxy stearate (using

What We Already Know about This Topic

• Diprivan, the currently available lipid-based emulsion propofol formulation, is effective, but has several drawbacks
• There have been a number of attempts to remedy these drawbacks
• Semifluorinated surfactant-based emulsions have been used for IV drug delivery

What This Article Tells Us That Is New

• Three propofol nanoemulsions prepared using novel semifluorinated surfactants were as effective and as potent as Diprivan in impairing the righting reflex of rats with bolus dosing, without apparent toxic effects
• A bolus of lipid emulsion accelerated clearance of propofol from its effect site after an induction dose of either lipid-based Diprivan or lipid-free fluoropolymer-based emulsions but was more effective for the lipid-free emulsion
no lipid), has become clinically available in some parts of the world.26–28

In this set of experiments, we studied three propofol nanoemulsions prepared using novel semifluorinated surfactants (L3, B8, and F8), and we compared their anesthetic effects to a formulation containing only the classical surfactant Lipoid E80 (L80) and to the clinically used lipid-based formulation (Diprivan). Semifluorinated surfactant-based emulsions have been studied as blood substitutes and also used for IV drug delivery, including IV delivery of the inhalational anesthetic sevoflurane.29–32 Semifluorinated surfactants were chosen for their unique architecture (lipophilic and fluorophilic blocks) and designed to eliminate the need for soybean oil in the emulsion. The lipophilic moiety was intended to stabilize the dissolved propofol (through van der Waals interactions) and the fluorophilic moiety to stabilize the nanodroplet emulsion (through solvophobic and lipophobic self-assembly). Due to the unique nature of the carbon–fluorine bond, perfluorocarbons—carbon chains fully saturated with bonds to fluorine—are both hydrophobic and lipophobic. This combined hydrophobicity and lipophobicity, called fluorophilicity, provides a unique driving force for the self-assembly of perfluorinated materials.31

In addition to testing these emulsions for stability and efficacy, we tested whether a postinduction bolus of lipid would accelerate recovery from the anesthetic effects of propofol, a highly lipid-soluble drug, as it does for the toxic effects of several other lipid-soluble drugs including bupivacaine.33 The rationale for these studies is that the octanol:water partition coefficient (log P) of propofol is 3.79,34 which makes it more lipid soluble than bupivacaine (log P 3.41).35 Therefore, a lipid bolus might reduce the effect-site concentration of propofol through partitioning or accelerated clearance, thereby reducing the duration of anesthesia.

Materials and Methods

All animal studies were approved by the University of Wisconsin Animal Care and Use Committee, Madison, Wisconsin, and were performed in accordance with the guidelines laid out in the Guide for the Care and Use of Laboratory Animals published by the National Research Council. Experiments were carried out in two phases. The purposes of the first phase were threefold (1) to test the efficacy of L3, B8, F8, and L80 in anesthetizing rats, as indicated by the detection limit of the instrumentation—with 1H- and 19F-nuclear magnetic resonance and matrix-assisted laser desorption and ionization mass spectrometry.

Emulsions

All emulsions were prepared with a propofol concentration of 10 mg/ml by combining the surfactant, additives, and propofol in water (with salt or glycerol for isotonicity). B8, L3, and M5diH10 surfactant solutions were prepared as 25 mg/ml solutions by direct dilution of lyophilized solid in sterile, normal saline solution to a total volume of 16.82 ml. The emulsion L80, containing only Lipoid E80, from Lipoid GmbH (Germany), was prepared by dissolution of Lipoid E80 at a concentration of 12 mg/ml in 16.82 ml double-distilled water with added glycerol for isotonicity. L3 also contained 12 mg/ml Lipoid E80. The F8 surfactant solution was prepared as a 16 mg/ml solution in 13.42 ml normal saline with 3.4 ml perfluorooctyl bromide (PFOB; Synquest Labs, USA). The solutions were sonicated until completely dissolved. A volume of 0.18 ml of 2.6-disisopropylphenol (Sigma-Aldrich Co., USA) was added to the polymer solutions for a total volume of 17 ml. The high-speed homogenizer (Power Gen 500; Fisher Scientific, USA) and the microfluidizer (model 110 S; Microfluidics Corp., USA) were first cleaned with 70 and 100% ethanol, followed by 70 and 100% methanol, and finally with three rinses of Millipore water (Milli-Q Integral; Merck KGaA, Germany). Once prepared, each emulsion mixture was then homogenized with the high-speed homogenizer for 1 min at 21,000 rpm at room temperature. The crude emulsion was then microfluidized for 1 min at 5,000 psi with the cooling bath kept at 10°C. The final emulsion was then filtered with a 30-nm diameter, 0.45-μm nylon filter and stored in 45-ml plastic centrifuge tubes (Corning Inc., USA) at 4°C.

An emulsion is considered stable if it does not immediately phase-separate after the emulsification procedure; the less the ripening (increase in particle size) that occurs over time, the more stable is the emulsion. After preparation and filtration of the emulsions, the emulsions were assessed for stability by measuring droplet sizes by dynamic light scattering (NICOOMP 380ZLS; Particle Sizing Systems, USA). An aliquot of the emulsion, approximately 150 μl, was diluted in 3 ml of Millipore water to achieve an intensity factor range of 300 to 350. Each measurement was run for 5 min at room temperature and repeated in triplicate. The data were analyzed by Gaussian analysis and reported as a volume-weighted average diameter. The emulsion errors for all polymers were taken as an average of the SDs of each individual measurement. The particle size of each stable emulsion formulation was monitored until either phase separation occurred or particles surpassed the 500-nm cutoff,
which is defined by United States Pharmacopeia monograph 729 as the shelf-life for emulsions stored at 4°C.

Animal Studies
Phase 1 and 2 experiments were carried out in six male Spraque–Dawley rats (Harlan Spraque-Dawley, Inc., USA) weighing approximately 280 g. The rats were received from the supplier with a surgically implanted jugular catheter. In all cases, the rats received only one dose of anesthetic per day with a minimum washout period of 20 h between administrations. Emulsions were prepared fresh at the beginning of each round of studies and were stored at 4°C during the study period. They were kept at room temperature during the approximately 4-h period each day when experiments were being performed.

In phase 1, experiments to measure LORR and recovery of righting reflex (RORR) were conducted using five different propofol formulations: (1) Diprivan, (2) L3, (3) B8, (4) F8, and (5) L80. For each of the first three formulations, five different doses (5, 6.25, 7.5, 10, and 15 mg/kg) were administered a minimum of five times each, based on prior studies that used a similar trial design to estimate the threshold dose for LORR in mice and rats. For F8, for which a limited amount of the fluoropolymer surfactant was available, each of the five doses was administered only three times. For L80, the highest dose (15 mg/kg) was tested six times. Since this dose did not lead to LORR, a limited number of lower doses were studied, and none led to LORR.

On any given experimental day, the emulsion to be tested was selected in a pseudorandom fashion, the primary criterion being that the same emulsion was not tested 2 days in a row. For a given rat, the dose to be administered was selected randomly, and the investigator scoring the animals for LORR and RORR was blinded to the dose but not to the emulsion being tested. As additional control studies, the L3, B8, and F8 fluoropolymers were injected alone without the propofol component three times each, at doses equivalent to those received in the 15 mg/kg experiments, in a separate group of three rats.

The propofol emulsions were administered by first weighing the rat and then restraining it with a towel. The plug placed at the end of the catheter was removed and replaced with a 23-gauge blunt tip needle connected to an insulin-type syringe. To remove the heparin-based fill solution and check that no blockage was obstructing the catheter, the syringe plunger was slowly withdrawn until blood filled the catheter. The 23-gauge blunt tip needle was then removed, and the catheter was connected using a 23-gauge connector tip to the tubing and syringe containing the propofol emulsion to be tested. The rat was placed in a transparent cage for observation. Forty microliters of the emulsion,
corresponding to the volume of the catheter, was injected to prime the catheter, and then the administration of the emulsion was started. The emulsion injection rate was controlled through an infusion pump (11 plus; Harvard Apparatus, USA). A bolus dose was delivered over 20 s regardless of the dose. LORR was evaluated by rolling the rat onto its back and observing whether the animal was able to right itself. The times to achieve and to recover from LORR were determined by the blinded observer. After recovery, rats were monitored by visual inspection for at least 15 min and up to 2 h to assess for adverse events including agitation, respiratory depression, qualitative changes in behavior, or death. All five doses of a single formulation were administered each day. If the effect of a given dose was equivocal (e.g., the animal continued to try to right itself throughout the period of observation, but not all four paws remained firmly planted), that dose was repeated with the sixth rat. This resulted in more data points for the lower doses near the estimated threshold dose. When a rat completely recovered from LORR, the catheter was flushed with 40 μl of 0.9% saline solution to remove the residual emulsion and then refilled with 40 μl of a heparin-based fill solution. The end of the catheter was then sealed with a sterile plug.

In phase 2, we measured the effect of a lipid bolus on the duration of anesthesia induced by a bolus of B8 or Diprivan. We elected to test B8 due to the simplicity of the emulsion and its uniqueness to Diprivan as it contained only the fluoropolymer surfactant plus propofol. For both propofol formulations, three different doses (7.5, 10, and 15 mg/kg) were administered five times each. These doses all reliably caused LORR with both emulsions, as expected from phase 1 results. In the same fashion as in phase 1, the rats were restrained and connected to the tubing and syringe containing the propofol emulsion. A bolus dose was administered, and 60 s after initiation of the bolus propofol dose, the animals’ catheters were connected to tubing and a syringe containing Intralipid (20% lipid emulsion; Baxter Healthcare Corp., USA). A bolus of lipid was then administered over 60 s. For the highest propofol dose administered (15 mg/kg), three different lipid bolus doses were administered five times each (3.75, 7.5, and 15 ml/kg). For the two lower doses of propofol (7.5 and 10 mg/kg), only the highest dose of lipid (15 ml/kg) was administered. Dosing was based on previously published data using lipid for treatment of drug toxicity in rats.33,41–44

Statistical Analysis

The threshold dose for causing LORR was defined as the x-intercept of the linear regression between duration of LORR and log dose for each emulsion.37 To compare x-intercepts for different emulsions, regression lines were fit to the aggregate data sets for the four emulsions, and statistical significance of the difference in threshold doses was determined using Student’s t test. Doses for which the majority of administrations did not cause LORR were excluded from linear regression analysis.

The slope of the linear regression line for each emulsion was used to represent the inverse clearance. A linear regression t test was used to compare slopes for the different emulsions. This method was also used to compare effects of a lipid bolus on Diprivan and B8. To evaluate the effect of changing the volume of a lipid bolus on the duration of anesthesia, each group was individually compared with “baseline” (i.e., no lipid administered) using Student’s t test.

The Benjamini–Hochberg method was used to correct for multiple comparisons (e.g., B8 vs. F8, B8 vs. L3, etc.). Adjusted P values were reported for all measures and were considered significant at a level of 0.05. The R software suite (http://www.r-project.org; accessed July 23, 2015) was used for all statistical analyses.

Results

Emulsion Stability

We investigated the ability of several novel semifluorinated surfactants to solubilize propofol (hydrophobic moiety) and stabilize the nanodroplet (fluorinated moiety). We found that the B8 formulation, containing only M1H10F8 surfactant and propofol dispersed in normal saline, formed a stable emulsion with an initial average particle size of approximately 150 nm but that increased at a rate of 3.6 nm/day until phase separation at 42 days of testing. A similar formulation using M1H10-O-F3 failed to produce a stable emulsion. However, the addition of Lipoid E80—the surfactant used in Diprivan—as an equimolar cosurfactant to M1H10-O-F3 formed a stable emulsion (L3) that grew at a rate of only 0.02 nm/day over 406 days of testing at which point no emulsion remained to test (fig. 2).

Lipoid E80 alone also formed a stable emulsion (L80) when glycerol instead of salt was used to achieve isotonicity. The emulsion was quite stable, with a growth rate of 0.50 nm/day until phase separation at 154 days. The classical (hydrophilic–lipophilic) surfactant M5diH10 was able
to emulsify propofol, but particle size increased rapidly, with a growth rate of 12.52 nm/day until particles grew beyond 500 nm in size at 21 days. The final emulsion investigated (F8) utilized a linear, semifluorinated surfactant M1H10F8, structurally similar to L3, but also incorporated a fluorinated stabilizer, PFDB, instead of a phospholipid cosurfactant. This emulsion was also stable, with a growth rate of 1.65 nm/day until phase separation at 119 days (fig. 3).

Phase 1: Emulsion Efficacy
We next tested the ability of the five emulsions to induce anesthesia, as indicated by LORR. In total, 120 experiments were conducted in phase 1. Three of these experiments involved technical errors with either the catheter or the pump, and nine involved control experiments using fluoropolymer without propofol, leaving 108 experiments for final analysis.

The L80 emulsion caused only mild sedation and failed to cause LORR up to a propofol dose of 15 mg/kg. The other four formulations (Diprivan, L3, B8, and F8) all proved effective at inducing LORR and were tested at doses of 5, 6.25, 7.5, 10, and 15 mg/kg.

The time to achieve LORR was measured from the start of the 20-s injection and was plotted as a function of the dose administered (fig. 4). Time decreased with increasing dose. Subjectively, there was little difference in the onset of anesthesia among the four emulsions that caused LORR. The ranges in times to achieve LORR for each emulsion were as follows: Diprivan, 8 to 22 s; L3, 9 to 23 s; B8, 9 to 24 s; and F8, 10 to 22 s.

The time to RORR, measured from the start of the 20-s injection, was also plotted as a function of the dose administered (fig. 5). Assuming first-order clearance kinetics, the threshold dose for inducing anesthesia was indicated by the x-intercept of the linear regression line on the semilog plot. Threshold doses for the four formulations were all similar, ranging from 5.8 to 8.6 mg/kg (table 1).

Statistical analysis showed that threshold dose did not differ for Diprivan versus B8 (Student’s t test, \( P = 0.067 \)) and that L3 was not different from B8 \( (P = 0.22) \) or F8 \( (P = 0.055) \), but the other three pairs (Diprivan/L3: \( P = 0.00022 \); Diprivan/F8: \( P = 0.00019 \); and B8/F8: \( P = 0.024 \) ) were significantly different. Notably, F8 proved to be effective at inducing anesthesia, but with prolonged duration at the highest dose (15 mg/kg).

The slopes of the linear regression lines were used to represent inverse clearance, with a lower slope indicating more rapid clearance or decreased availability at the effect site (faster RORR for a given dose). As shown in table 1, there were no significant differences between the slopes of the linear regression lines (Diprivan/L3: linear regression \( t \) test, \( P = 0.67 \); Diprivan/B8: \( P = 0.098 \); L3/B8: \( P = 0.076 \); L3/F8: \( P = 0.054 \); Diprivan/F8: \( P = 0.066 \); and B8/F8: \( P = 0.054 \)). No ill effects were seen in the rats acutely or after more than 10 doses over the study period.

As control studies, we tested whether the fluoropolymers administered alone (i.e., without propofol) caused any effects. We observed no signs of anesthesia, or any other behavioral changes, during or after administration of the fluoropolymers.

Phase 2: Lipid Studies
In phase 2, totaling 57 additional experiments, we tested whether a bolus of lipid emulsion would increase clearance and accelerate recovery after a bolus dose of propofol. Thus, we administered 7.5, 10, or 15 mg/kg Diprivan or B8, followed immediately by a bolus of 15 ml/kg lipid. The time to RORR was plotted as a function of propofol dose, with or without a lipid bolus. The slope of the linear regression line was used to represent inverse clearance, again assuming first-order clearance kinetics (fig. 6 and table 2). We found that...
this lipid bolus significantly accelerated clearance of propofol, for both Diprivan and B8 formulations.

To test whether a smaller lipid bolus would also accelerate recovery, we administered the highest dose of propofol (15 mg/kg of B8 and Diprivan) in combination with two lower doses of lipid (7.5 and 3.75 ml/kg). Only the 15 ml/kg lipid dose caused a significant reduction in duration of anesthesia for B8 \( (P = 0.0023) \), and no lipid dose caused a significant reduction in duration of anesthesia for Diprivan (fig. 7).

**Discussion**

Our major finding was that the three novel fluoropolymer-based emulsions of propofol (L3, B8, and F8) were all able to reliably induce anesthesia in rats. There were no ill effects either acutely or after more than 10 administrations over a 2- to 3-week period. In addition, the threshold doses for the lipid-free emulsions were similar to the lipid-emulsified Diprivan. Although there were some statistically significant
differences in threshold doses and clearance rates, they were of small enough magnitude that the differences may be of little clinical significance.

In the formulation of stable nanoemulsions, emulsions whose particle sizes are less than 500 nm, Brownian motion is able to prevent creaming, sedimentation, and flocculation. Ostwald ripening—the diffusion of dissolved oil phase from small droplets to larger droplets—becomes the single mechanism leading to particle size increase. Once particles reach a large enough size (more than 500 nm), they are no longer useful for IV administration, and at larger sizes, the emulsion can phase-separate. To reduce Ostwald ripening, a second, less-soluble oil additive may be added to emulsions (like the added soybean oil in Diprivan). Removing this oil additive can lead to unstable emulsions (like the M5diH10 emulsion; fig. 3).

Fluorinated surfactants were selected for their potential to create stable nanodroplets without any oil additive. Furthermore, fluorocarbons are highly resistant to physical and chemical degradation, and the fluorosurfactant architecture includes only carbon–carbon bonds and ether linkages, which are known to resist metabolism in vivo. For the B8 formulation, the fluorinated moiety and hydrocarbon chain are independent, and the emulsion formulation was stable without any additives. For the L3 formulation, the fluorinated surfactant has sequential fluorinated and hydrocarbon components. The initial formulation contained no Lipoid E80 additive and was unstable possibly due to the repulsive interaction between propofol and fluorphilic components. Lipoid E80 was added for its greater lipophilicity. It was found that only a 1:1 molar ratio of fluorinated and Lipoid E80 surfactants ultimately led to the stable L3 formulation. Finally, a larger fluorphilic component was found to provide a stable formulation (F8) with the addition of PFOB. This was devised as a fluorous emulsion—fluorinated nanodroplets solubilized by a semifluorinated surfactant—where the propofol would be solubilized in the intermediate lipophilic shell. The F8 formulation was developed out of concern that the B8 and L3 emulsion might too slowly release propofol, while F8 might release propofol faster, with propofol residing in an intermediate shell.

Interestingly, the L80 formulation, containing only propofol and Lipoid E80, did not cause LORR even at a high dose of 15 mg/kg. The reduced effect-site availability of propofol from the L80 formulation could be a result of the self-assembly properties of the phospholipid surfactant coupled with the small volume of propofol. Phospholipids are known to form vesicles and bilayers. Given the relatively small volume of propofol (0.18 ml in 17 ml of water), it is possible that instead of a typical nanoemulsion—nanodroplets solubilized by the surfactant—we have produced propofol-loaded vesicles/bilayers. These aggregates will be characterized by different release properties than the typical nanoemulsion nanodroplets.

A second important finding was that clearance of propofol from its effect site can be accelerated with a lipid bolus after an induction dose. This effect was observed as a change in threshold doses and clearance rates, they were of small enough magnitude that the differences may be of little clinical significance.

**Table 2. Influence of Lipid Bolus on Drug Clearance**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Slope ± SEM (s)</th>
<th>Slope-lipid ± SEM (s)</th>
<th>P Value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diprivan</td>
<td>1,602 ± 84</td>
<td>1,030 ± 202</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>B8</td>
<td>1,250 ± 178</td>
<td>602 ± 94</td>
<td>0.0054</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The slope of the linear regression represents the inverse clearance, for Diprivan and B8, with and without lipid bolus (fig. 6). Sample sizes: Diprivan (16); Diprivan-lipid (17); B8 (16); and B8-lipid (17).

Sig = statistical significance.
in the slope of the duration versus dose in the presence of lipid (fig. 6) and was most pronounced when a high dose of drug (15 mg/kg) was followed by a high volume of lipid (15 ml/kg). Several mechanisms have been proposed for lipid rescue in bupivacaine toxicity and other highly lipid-soluble drugs. A commonly cited mechanism is “partitioning,” in which lipid acts as an intravascular “sink,” causing decreased effect-site concentrations. Partitioning has been proposed as a mechanism for several lipid-soluble drugs (local anesthetics, calcium channel blockers, β-blockers, etc.) whose toxicity has been treated with lipid infusion.49,50 This mechanism is consistent with our results, as propofol is more lipid soluble than bupivacaine.34,35 A second (related) proposed mechanism is accelerated shunting of the drug to its site of metabolism, typically the liver for lipid-soluble drugs.33,46,47 In either case, there is increased clearance of the drug from the effect site (i.e., γ-aminobutyric acid type A receptors in the central nervous system). Additional proposed mechanisms for beneficial effects of lipid rescue on cardiovascular function are a direct effect of lipid itself on cardiac muscle cell function and a reversal of inhibition of fatty acid metabolism in cardiac muscle.46 In a related manner, it is possible that lipid itself interferes with propofol binding to the γ-aminobutyric acid type A receptor and that there is a threshold concentration required to see this effect, which the 7.5 and 3.75 ml/kg doses were not large enough to reach. However, we have no evidence in favor of this possibility, and it remains speculation.

Lipid reversal was more pronounced for B8 than for Diprivan. A possible explanation for this finding may be that the effect-site concentration of propofol is already limited to some extent by the lipid component of Diprivan, and the additional lipid does not augment clearance as much as it does for the lipid-free formulation.

The 15 ml/kg dose of lipid that was found to be effective in this study is quite large and would not be clinically useful in humans if this volume is required to accelerate recovery from a bolus dose or prolonged infusion of propofol. However, it is possible that lower volumes would be effective in humans compared with rats. Induction of anesthesia with propofol in humans typically requires 1 to 2 mg/kg, but in rats that dose is 5 to 10 times higher. Similarly, bupivacaine toxicity in the rat requires 13.5 ml/kg of 30% lipid emulsion,33 whereas recommended doses in humans are 1.5 ml/kg bolus followed by 0.25 to 0.5 ml·kg⁻¹·min⁻¹ 20% lipid emulsion for 10 min (total 4 to 6 ml/kg).46

Although we studied the effect of lipid bolus after administration of an induction dose of propofol, there may be greater clinical utility for lipid reversal after a prolonged infusion of propofol compared with a bolus. This possibility merits evaluation in a future study, particularly if circulating lipid during Diprivan infusion sufficiently accelerates propofol clearance so that little effect would be seen from an additional lipid bolus. A lipid-free propofol emulsion may have decreased clearance during infusion and therefore require lower infusion rates, in which case lipid reversal may have an even more substantial effect.

There are several limitations of this study that should be discussed. First, the use of linear regression to estimate threshold dose as well as clearance assumes that the pharmacokinetics of the drugs can be described by a one-compartment model, and this is unlikely to be the case. Using a multicompartment model may thus change the estimated threshold doses and drug clearance. Second, although no ill effects were seen in our study, for further development of these emulsions, in-depth pharmacokinetic and toxicity studies, as well as studies to address venous irritation, pain on injection, microbial growth, and hyperlipidemia, which are the primary weaknesses of the currently available propofol formulation, will be required. Third, the observer determining LORR and RORR was not blinded to the specific emulsion being tested. The observer was blinded to dose, but due to the differences in visual appearance of the emulsions, it was impractical to blind the observer to emulsion using the personnel and equipment available. Finally, the method used to determine RORR involved the rat spontaneously turning itself from the supine to the prone position. Using a different endpoint, for example, movement in response to painful stimulus might have produced different results.

In summary, the three lipid-free fluoropolymer-based formulations of propofol all showed similar efficacy and potency in producing and maintaining anesthesia with bolus dosing comparable to Diprivan. Additionally, clearance of propofol from its effect site was accelerated with lipid after an induction dose using either lipid-based Diprivan or lipid-free fluoropolymer-based emulsions, but more substantially for the lipid-free emulsion. These lipid-free formulations have the potential to avoid complications related to microbial growth and hyperlipidemia that are seen with the currently available formulation of...
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propofol, and their effects may, to a certain extent, be reversible with lipid infusion. Further study is indicated to determine toxicity and side effect profiles of these novel surfactant formulations before they can be considered for clinical use.

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Competing Interests

Patent applications have been submitted for B8, L3, and F8, the fluoropolymer-based emulsions described in this study.

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