The Use of Dye Surrogates to Illustrate Local Anesthetic Drug Sequestration by Lipid Emulsion

A Visual Demonstration of the Lipid Sink Effect

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Background and Objectives: We hypothesized that by substituting a dye surrogate in place of local anesthetic, we could visually demonstrate dye sequestration by lipid emulsion that would be dependent on both dye lipophilicity and the amount of lipid emulsion used.

Methods: We selected 2 lipophilic dyes, acid blue 25 and Victoria blue, with log *P* values comparable to lidocaine and bupivacaine, respectively. Each dye solution was mixed with combinations of lipid emulsion and water to emulate "lipid rescue" treatment at dye concentrations equivalent to fatal, cardiotoxic, and neurotoxic local anesthetic plasma concentrations. The lipid emulsion volumes added to each dye solution emulated equivalent intravenous doses of 100, 500, and 900 mL of 20% Intralipid in a 75-kg adult. After mixing, the samples were separated into a lipid-rich supernatant and a lipid-poor subnatant by heparin flocculation. The subnatants were isolated, and their colors compared against a graduated dye concentration scale.

Results: Lipid emulsion addition resulted in significant dye acquisition by the lipid compartment accompanied by a reduction in the color intensity of the aqueous phase that could be readily observed. The greatest amount of sequestration occurred with the dye possessing the higher log *P* value and the greatest amount of lipid emulsion.

Conclusions: Our study provides a visual demonstration of the lipid sink effect. It supports the theory that lipid emulsion may reduce the amount of free drug present in plasma from concentrations associated with an invariably fatal outcome to those that are potentially survivable.

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Local anesthetic systemic toxicity is an important life-threatening emergency for which intravenous lipid emulsion (ILE) therapy has been successfully used.[1,2] Lipid emulsion also appears to be effective in the reversal of other lipophilic drug overdoses, and guidance has also been published supporting its use in their treatment.[3–6] Several mechanisms may explain the effect of ILE. One mode of action is based on the "lipid sink theory," whereby administration of ILE creates an intravascular lipid-rich compartment distinct from the plasma aqueous phase with the capacity to sequester lipophilic drugs, thus preventing their harmful toxic effects.[7–12] Lipid emulsion provides a source of fatty acids that may increase the intramitochondrial transfer of acylcarnitines, thereby facilitating metabolism governed by oxidative phosphorylation.[13] In addition, the long-chain fatty acids of lipid emulsion activate voltage-dependent calcium channels and increase cardiac intramyocyte calcium levels, resulting in a positive inotropic effect.[14] These mechanisms may contribute to the reversal of manifestations of drug toxicity either individually or, possibly, in combination.

The lipid sink theory is an attractive concept but is difficult to demonstrate in view of the specific laboratory requirements necessary to determine drug plasma concentrations in both the aqueous and lipid phases. Direct drug measurement in the presence of hyperlipidemia or significant exogenous lipid emulsion is complex and usually requires specialized methods, including ultrafiltration and equilibrium dialysis or immobilized liposome electrokinetic capillary chromatography; these techniques are not universally accessible.[15–17]

Bearing in mind the complexities of analysis of drug concentrations in lipid-rich samples, we undertook to emulate the lipid sink effect using dyes with *n*-octanol/water partition coefficients (also described as log *P* values) analogous to 2 local anesthetics.[18] We predicted that dye sequestration would be dependent on the amount of lipid emulsion added and the lipid solubility of the dye (acting as a surrogate for its respective local anesthetic). Our study was designed to provide a visual demonstration of the lipid sink effect created by addition of lipid emulsion without the need for specific laboratory drug assays.

METHODS

Dye Sample Preparation

We identified 2 dyes: acid blue 25 (AB25) (FastColours, Huddersfield, UK) with a log *P* value of 2.22 as a surrogate for lidocaine (log P 2.36) and Victoria blue B (VB) (VWR International Ltd, Poole, UK) with a log *P* value of 3.28 as a bupivacaine surrogate (log P 3.64).[19–21] Stock solutions of AB25 were prepared at concentrations of 321, 540, and 961 μmol/L. Similarly, solutions of VB were prepared at concentrations of 57, 98, and 173 μmol/L. These solutions were used as the basis for subsequent combination with lipid emulsion. We used 20% Intralipid (Fresenius Kabi, Runcorn, Cheshire, UK) in our studies, according to the methodology outlined in the experimental design section.

Experimental Design

The study of each dye required 9 burettes: these burettes were subdivided into 3 separate groups representing

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differing levels of local anesthetic systemic toxicity as follows (Table 1)

- Fatal Dose Surrogates. Two sets of burettes labeled 1, 4, and 7 each received 8 mL of stock dye solutions containing AB25 and VB concentrations of 961 and 173 μmol/L, respectively. Taking into account the dilutional effect of the volumes of other additives used in the study, this would result in final dye concentrations of approximately 641 and 115 μmol/L, respectively, approximating the equivalent molar concentrations of their respective local anesthetic that are universally lethal.[22]
- Cardiotoxic Surrogates. Two sets of burettes labeled 2, 5, and 8 each received 8 mL of stock dye solutions containing AB25 and VB at concentrations of 540 and 98 μmol/L, respectively. Similarly, when the dilutional effects of other additives necessary for the study were acknowledged, this would result in final dye concentrations of approximately 360 and 65 μmol/L, approximating the equivalent molar concentrations of their respective local anesthetic counterparts known to cause cardiac arrhythmias/arrest.
- Neurotoxic Surrogates. Two sets of burettes labeled 3, 6, and 9 each received 8 mL of stock dye solutions containing AB25 and VB at concentrations of 321 and 57 μmol/L, respectively. When the dilutional effects of the other additives required for the study were taken into account, this would result in final dye concentrations of approximately 214 and 38 μmol/L. These dye concentrations represent equivalent molar concentrations of their respective local anesthetic counterparts that will cause convulsions.[23]

Three lipid emulsion treatment groups were allocated as follows. Depending on the volume of lipid emulsion added, equal final volumes and hence consistent dilution for all burettes were maintained by adding distilled water (Table 1):

- 100 mL ILE equivalent treatment group. Burettes 1, 2, and 3 received 0.3 mL 20% lipid emulsion and 2.7 mL distilled water.
- 500 mL ILE equivalent treatment group. Burettes 4, 5, and 6 received 1.5 mL 20% lipid emulsion and 1.5 mL distilled water.
- 900 mL ILE equivalent treatment group. Burettes 7, 8, and 9 received 2.7 mL 20% lipid emulsion and 0.3 mL distilled water.

All samples were mixed thoroughly.

Lipid-Aqueous Phase Separation

One milliliter (25,000 IU) of preservative-free, neutral-pH heparin (Wockhardt, Wrexham, UK) was added to each sample, which was vigorously mixed. Hence, all burettes contained a total final volume of 12 mL (Table 1). All burettes were allowed to stand for 4 hrs at room temperature (21°C). Addition of heparin to lipid suspensions promoted flocculation and resulted in a distinct separation of a lipid-rich compartment (a supernatant containing >70% of the triglyceride component of the lipid emulsion) from the aqueous phase (subnatant).[24,25] Once separation was complete, the subnatant was carefully drained from the burette. Each dye solution was placed in an evenly illuminated transparent cuvette, and a digital photographic image (Canon EOS 1000D SLR digital camera) was obtained for comparison with surrogate local anesthetic dye concentration color scales prepared as described in the following section.

Surrogate Local Anesthetic Dye Concentration Color Scales

An incremental 20-point dye color scale containing a uniform low-level lipid suspension of approximately 0.5% was created.[24,25] Triglyceride concentrations of this order remain in suspension in the aqueous phase, even after flocculation, and are known to cause an optical scattering effect.[26] The dye color scale was created as follows: First, a 5% lipid suspension was flocculated using heparin, and the resulting subnatant collected. This subnatant was mixed with an equal volume of surrogate dye solutions, creating dye solutions with a lipid content mirroring the concentration of the subnatant obtained following flocculation of a 2.5% (500-mL dose) lipid suspension. Thereafter, additional dye dilutions were produced by using increasing volumes of the subnatant of a flocculated 2.5% lipid suspension. The final dye concentration color scale for each dye surrogate ranged from the equivalent fatal local anesthetic concentration to 1/20th of this concentration (Figs. 1 and 2). Each dye solution was placed in a cuvette, and a digital photographic image was obtained using an identical method to the test samples.

Visual comparison of the best color match between each treatment group subnatant and the dye concentration color

TABLE 1. Experimental Design of the Surrogate Dye Samples at 3 Different Levels of Local Anesthetic Toxicity and 3 Lipid Emulsion Treatment Groups

Treatment Group	Dye Volume, mL	Predilutional Dye Concentration, μmol/L	Intralipid Volume, mL	Water Volume, mL	Heparin Volume, mL	Total Volume, mL	Postdilutional Dye Concentration, μmol/L	Intralipid Concentration, mg/mL
Intralipid 100 mL	8	AB25: 321 (N), 540 (C), 961 (F) VB: 57 (N), 98 (C), 173 (F)	0.3	2.7	1	12	AB25: 214 (N), 360 (C), 641 (F) VB: 38 (N), 65 (C), 115 (F)	5
Intralipid 500 mL	8	AB25: 321 (N), 540 (C), 961 (F) VB: 57 (N), 98 (C), 173 (F)	1.5	1.5	1	12	AB25: 214 (N), 360 (C), 641 (F) VB: 38 (N), 65 (C), 115 (F)	25
Intralipid 900 mL	8	AB25: 321 (N), 540 (C), 961 (F) VB: 57 (N), 98 (C), 173 (F)	2.7	0.3	1	12	AB25: 214 (N), 360 (C), 641 (F) VB: 38 (N), 65 (C), 115 (F)	45

N indicates neurotoxic concentration; C, cardiotoxic concentration; F, fatal concentration.

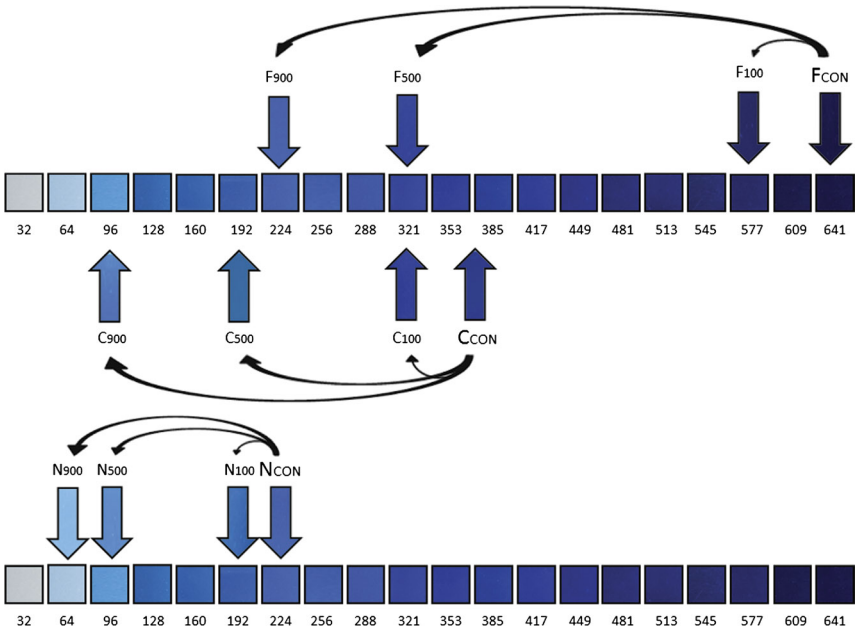

FIGURE 1. Lidocaine-dye surrogate concentration scale, demonstrating the reduction in color intensity for fatal (F), cardiotoxic (C), and neurotoxic (N) doses of lidocaine. Each arrow indicates the color obtained following the use of 20% lipid emulsion equivalent to 100-, 500-, and 900-mL volumes, respectively. Key: (F or C or N)$_{CON}$ = control groups, (F or C or N)$_{100}$ = 100 mL equivalent treatment group, (F or C or N)$_{500}$ = 500 mL equivalent treatment group, (F or C or N)$_{900}$ = 900 mL equivalent treatment group.

scale was undertaken independently by 2 of the authors (A.P. and T.L.S.).

RESULTS

Following flocculation, the lipid-rich supernatant revealed significant visible dye acquisition at every dye sample concentration; conversely, a reduction of color intensity in the subnatant compared with the initial dye solutions was also immediately apparent. A greater reduction in dye intensity of the subnatant was observed when larger amounts of lipid emulsion had been added to the original dye solution. A greater visual difference, before and after addition of equivalent volumes of lipid

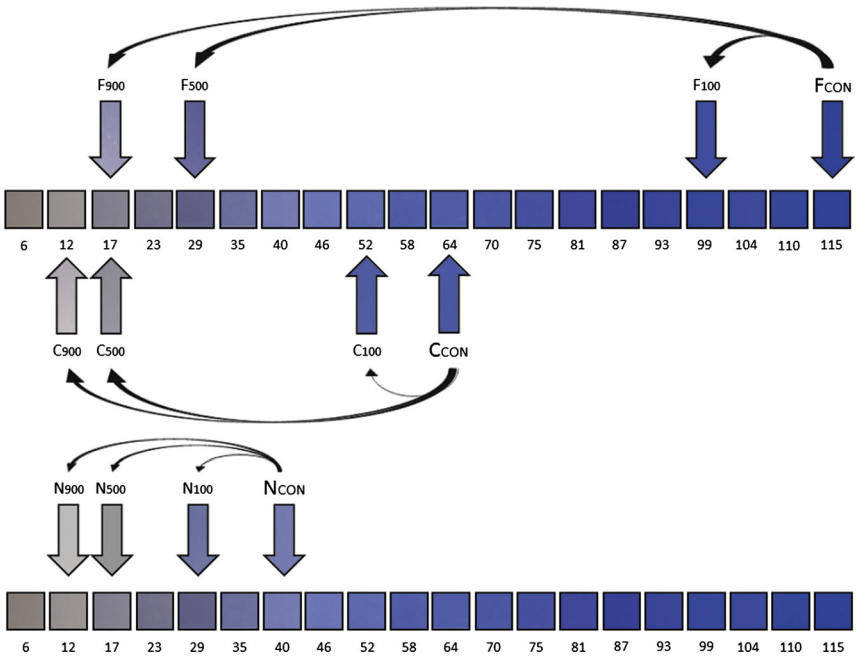

FIGURE 2. Bupivacaine-dye surrogate concentration scale, demonstrating the reduction in color intensity for fatal (F), cardiotoxic (C), and neurotoxic (N) doses of lidocaine. Each arrow indicates the color obtained following the use of 20% lipid emulsion equivalent to 100-, 500-, and 900-mL volumes, respectively. Key: (F or C or N)$_{CON}$ = control groups, (F or C or N)$_{100}$ = 100 mL equivalent treatment group, (F or C or N)$_{500}$ = 500 mL equivalent treatment group, (F or C or N)$_{900}$ = 900 mL equivalent treatment group.

emulsion, was evident when comparing the VB (bupivacaine surrogate) group to the AB25 (lidocaine surrogate) group consistent with the greater lipid solubility of VB dye (Figs. 1 and 2).

Fatal Dose Surrogates

Addition of lipid emulsion to the dye preparations representing the most toxic local anesthetic concentration equivalents reduced the subnatant lidocaine surrogate (AB25) dye concentration from an initial value of 641 μmol/L to approximately 577, 321, and 224 μmol/L, with increasing equivalent ILE treatment doses of 100, 500, and 900 mL, respectively. This effect was replicated to a greater degree with the VB surrogate dye samples, when the subnatant concentrations decreased from the initial value of 115 μmol/L to approximately 99, 29, and 17 μmol/L, respectively.

Cardiotoxic Surrogates

Analogous reductions in surrogate dye concentrations occurred with lipid emulsion treatment of the higher dye concentrations, approximating cardiotoxic concentrations of local anesthetics. Lipid emulsion reduced the subnatant lidocaine surrogate (AB25) dye concentration from an initial value of 360 μmol/L to approximately 321, 192, and 96 μmol/L, and the bupivacaine surrogate (VB) concentration from an initial value of 65 μmol/L to approximately 52, 17, and 12 μmol/L with increasing equivalent ILE doses of 100, 500, and 900 mL.

Neurotoxic Surrogates

Visual comparison of the colors of the AB25 and VB dye subnatants after lipid emulsion treatment with their respective dye concentration color scales indicated a reduction in the lidocaine surrogate dye (AB25) subnatant concentration, from an initial value of 214 μmol/L to approximately 192, 96, and 64 μmol/L with increasing lipid emulsion volumes equivalent to treatment doses of 100, 500, and 900 mL. The same lipid volumes decreased the bupivacaine surrogate (VB) subnatant concentration from an initial value of 38 μmol/L to approximately 29, 17, and 12 μmol/L, respectively.

DISCUSSION

We have demonstrated through visual comparison of surrogate local anesthetic dye solutions that addition of larger volumes of lipid emulsion reduces the color intensity of the aqueous phase, an effect that is most marked by the surrogate local anesthetic dye solution possessing the greatest lipid solubility.

The volumes of lipid emulsion and water used represent corresponding treatment volumes of 100, 500, or 900 mL of 20% lipid emulsion combined with crystalloid resuscitation of 1000 mL administered to a 165-lb patient. Previously, the dilutional effect alone has been postulated to explain the reduction in plasma concentration of drugs originally present at toxic levels.[27] Our experimental design appears to refute this theory because the dilutional effects of lipid emulsion, water, and heparin were constant throughout the study. By doing this, we accommodated any dilutional effects, and our study provides compelling visual evidence of a specific dose-dependent ILE effect consistent with the lipid sink theory.

There are limitations to our study. First, using the same dye dilution scale for all 3 equivalent treatments groups introduced a small color comparison error. Nevertheless, we consider these differences to be so striking that even if they have been under or overestimated, the lipid sink effect is clearly demonstrated.

Second, the toxic concentrations used were based on animal studies because of lack of consistent data on human subjects.[28,29] Dye concentrations representing equivalent local anesthetic values greater than those known to produce toxicity were selected. Nonetheless, we acknowledge that clinical manifestations of local anesthetic toxicity depend on many variables. Drug binding in plasma is known to be complex, and carriage of lipophilic drugs is often facilitated by albumin or other plasma proteins. We are not able to comment on the potential interaction and relative affinities for drug binding between the surrogate local anesthetic dyes and plasma proteins.

Finally, our observations did not acknowledge the small quantities of lipid particles that remained suspended in the aqueous phase. These lipid particles constitute part of the lipid sink and thus sequester dye; however, this assertion contributes to an underestimation of the amount of dye sequestration by ILE. Furthermore, the log *P* values of both dye surrogates are marginally less than the local anesthetics they represent. Both these observations imply that the degree of binding of ILE for lidocaine and bupivacaine would be slightly greater than these surrogate dyes illustrate.

According to our results, the greatest reduction in color intensity for both dyes is observed when the equivalent lipid emulsion dose of 500 mL or more is administered. The increasing distances between the ILE treatment groups and their respective controls provide evidence to support a dose-dependent effect of ILE (Figs. 1 and 2). Direct extrapolation to clinical practice suggests that the greatest amount of lipid emulsion (currently believed to be 10–12 mL/kg) should be administered that is safe to do so.[1,4]

Significant decreases in free drug concentrations have been reported with the use of lipid emulsion both in the presence of local anesthetics and other lipophilic drugs in human plasma samples and animal studies, although to our knowledge there are no data regarding local anesthetic concentration reduction in vivo in humans.[8,9,17,30,31] Future studies to quantify the plasma levels attained before and after the use of ILE in lipophilic drug overdoses would identify drug toxidromes amenable to treatment with ILE and enhance development of optimum treatment protocols.

The effectiveness of ILE therapy appears to be governed by drug lipophilicity and lipid emulsion dose. Our study implies that a 500-mL dose of 20% lipid emulsion has the capacity to reduce a fatal plasma lidocaine and bupivacaine concentration to that below cardiotoxic/neurotoxic levels, which is potentially survivable. Our findings may suggest that other drugs with comparable lipophilicity and ionization will be subject to sequestration in a lipid sink to the same extent. Therefore, lipid emulsion treatment involving such lipophilic drug overdoses may have a similar potential benefit.

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