

Validity of the Lipid Sink as a Mechanism for the Reversal of Local Anesthetic Systemic Toxicity

A Physiologically Based Pharmacokinetic Model Study

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ABSTRACT

Background: *In vitro* observations support the lipid sink theory of therapeutic action by confirming the capacity of lipid emulsions to successfully uptake bupivacaine from aqueous media. However, competing hypotheses and some *in/ex vivo* small animal studies suggest that a metabolic or positive inotropic effect underlies the dramatic effects of lipid therapy. Controlled clinical tests to establish causality and mechanism of action are an impossibility. In an effort to quantitatively probe the merits of a “sink” mechanism, a physiologically based pharmacokinetic model has been developed that considers the binding action of plasma lipid.

Methods: The model includes no fitting parameters and accounts for concentration dependence of plasma protein and lipid:anesthetic binding as well as the metabolism of the lipid scavenger. Predicted pharmacokinetics were validated by comparison with data from healthy volunteers administered a nontoxic dose of bupivacaine. The model was augmented to simulate lipid therapy and extended to the case of accidental IV infusion of bupivacaine at levels known to cause systemic toxicity.

Results: The model yielded quantitative agreement with available pharmacokinetic data. Simulated lipid infusion following an IV overdose was predicted to yield (1) an increase in total plasma concentration, (2) a decrease in unbound concentration, and (3) a decrease in tissue content of bupivacaine.

Conclusions: Results suggest that the timescale on which tissue content is reduced varies from organ to organ, with

What We Already Know about This Topic

- IV administered lipid emulsion can resuscitate patients experiencing local anesthetic systemic toxicity
- Physiologically based pharmacokinetic models can test the hypothesis that the mechanism by which lipid emulsion resuscitates patients is by increasing local anesthetic IV solubility (the lipid sink hypothesis)

What This Article Tells Us That Is New

- Lipid emulsion was predicted to reduce heart tissue bupivacaine concentration by 11% within 3 min of initiating therapy and brain concentration by 18% within 15 min
- The lipid sink is not the sole mechanism by which IV administered lipid emulsion reverses local anesthetic systemic toxicity

the concentration in the heart falling by 11% within 3 min. This initial study suggests that, in isolation, the lipid sink is insufficient to guarantee a reversal of systemic toxicity.

IV lipid emulsions (ILEs) show increasing promise as mitigators of systemic toxicity due to lipophilic drug overdose.^{1–4} The often-cited theory regarding their method of action is known as the lipid “sink.” It is thought that when administered intravenously, lipid droplets exist as a discrete hydrophobic phase in the bloodstream into which lipophilic molecules preferentially partition. The sequestering of these pharmacologically active molecules is thought to allow pharmaceutical agents to be redistributed from tissues of critical organs such as the heart and brain to the bloodstream.

In an effort to quantitatively probe the possible merits of a sink mechanism, a physiologically based pharmacokinetic (PBPK) model has been developed. The PBPK model includes no fitting parameters, but rather draws primarily on experimentally determined parameters from the clinical and pharmacokinetic literature. Critically, the model accounts for concentration dependence of both plasma protein and lipid:anesthetic binding as well as the metabolism of the lipid scavenger—which occurs on a shorter timescale than the metabolism of the anesthetic. Also addressed is the partitioning of anesthetic into erythrocytes. The system of differential equations governing the systemic distribution of the anesthetic consists of mass balances based on

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the following assumptions: (1) clearance of the anesthetic occurs *via* hepatic metabolism⁵⁻⁸; (2) tissue disposition is perfusion limited; and (3) anesthetic in the bloodstream is assumed to be partitioned *via* rapid equilibrium between four subcompartments: bound to plasma proteins, bound to erythrocytes, bound to lipid droplets, or unbound in the aqueous plasma.

Predicted pharmacokinetics are validated by comparison with clinical data from healthy volunteers administered nontoxic doses of bupivacaine intravenously.^{6,9-11} Key pharmacokinetic quantities such as half-lives and steady-state volume of distribution were well reproduced. Predictions were also compared with those obtained by adopting the assumptions of a previously reported model of bupivacaine pharmacokinetics. This model, which does not account for the concentration-dependent nature of plasma protein binding, was found to overestimate tissue disposition.

The PBPK model was subsequently extended to mimic the case of accidental IV infusion at levels known to cause toxicity. ILE therapy was simulated according to existing guidelines and the consequent effects on tissue concentration were analyzed.

Materials and Methods

The PBPK model was developed in-house and implemented in Fortran 2008. It includes 14 compartments (fig. 1)

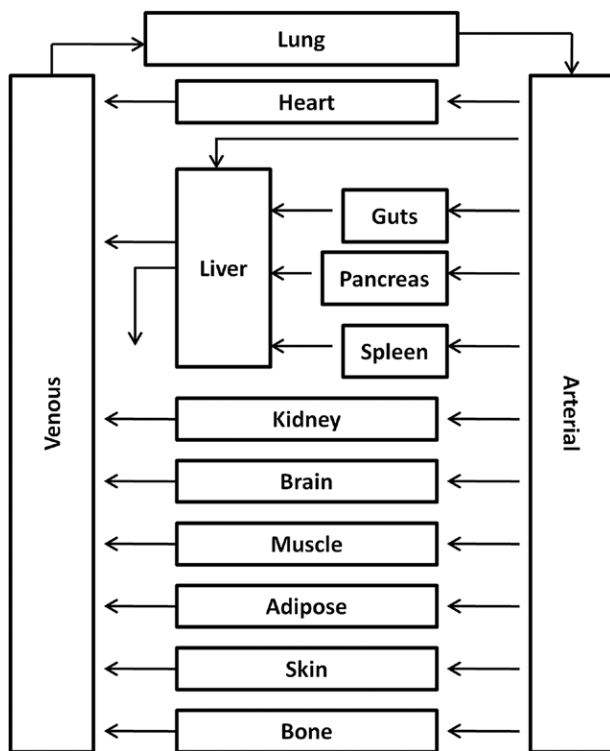


Fig. 1. Structure of the physiologically based pharmacokinetic model. Twelve organs and two blood compartments are represented in this 14-compartment model.

corresponding to 12 organs and two blood compartments (one arterial and one venous). Details of the governing differential equations are provided in the appendix.

Plasma Protein Binding

At therapeutic concentrations, amide drugs such as bupivacaine are ≈96% bound in the blood stream.^{6,7,12,13} An appropriate model for saturable uptake by a single class of plasma proteins is as follows:

$$\frac{C_{b,p}}{C_{f,p}} = \frac{np}{K_d + C_{f,p}} = K_{bf} \quad [1]$$

where np is the total binding capacity, K_d is the dissociation constant (inverse of affinity), and K_{bf} is the partition coefficient describing the relationship between $C_{b,p}$ and $C_{f,p}$, the protein bound and free concentration of anesthetic in the plasma, respectively. Bupivacaine has been observed to bind with two distinct sites in human serum, a low-affinity, high capacity site (human serum albumin) and a high-affinity, low capacity site (α -1 acid glycoprotein).¹⁴⁻¹⁶ Assuming that each of these interacts with the unbound anesthetic independently, their partition coefficients are additive:

$$\frac{C_{b,p}}{C_{f,p}} = \sum_j \frac{[np]_j}{[K]_j + [C_{f,p}]_j} = K_{bf} \quad [2]$$

Erythrocyte Partitioning

Data describing bupivacaine–erythrocyte partitioning is rare in the literature, as bupivacaine binding is often measured in plasma rather than whole blood. One approach to modeling erythrocyte partitioning is to employ an erythrocyte:plasma partition coefficient ($K_e = C_e/C_{f,p}$) estimated from reported blood:plasma ratios ($\lambda = C_{\text{blood}}/C_p$).¹⁷ An alternative approach is employed here. Tucker *et al.*⁹ quantified plasma:erythrocyte anesthetic distribution in blood samples from two healthy individuals. Partitioning was measured *in vitro* for bupivacaine concentrations in whole blood ranging from 7 to 70 μM ; the blood:plasma ratio was observed to vary in a nonlinear fashion from 0.56 to 0.83. Consequently, we chose not to use the blood:plasma ratio as a basis for determining the erythrocyte–plasma partitioning. A suitable model for erythrocyte partitioning allows for both transmembrane partitioning into intracellular water and binding to the cell membrane as follows.¹⁸

$$\frac{C_e}{C_{f,p}} = \left[I + \frac{B_{max,e}}{K_d + C_{f,p}} \right] = K_e \quad [3]$$

where C_e is the concentration of drug in the hematocrit and I describes the relationship between the aqueous intracellular portion of C_e and the free concentration in the plasma

(indicating the effect of differences in intracellular and extracellular pH). Data from the Tucker study yielded an apparent linear relationship between free bupivacaine in plasma and erythrocyte-associated bupivacaine, suggesting either a dominance of transmembrane partitioning (governed by I) or $K_d \gg C_{f,p}$ (giving $K_e \approx I + B_{\max,e}/K_d$). A linear fit to the observations of Tucker provided an estimate of the erythrocyte partition coefficient ($K_e = 1.37$ [95% CI, 1.303–1.436]). Data at higher blood concentrations would be required for independent determination of I , $B_{\max,e}$, and K_d . On this basis, the final relationship between whole blood and unbound drug concentrations can be shown to obey Equation [4], where H is the hematocrit and C_{blood} is the bupivacaine concentration in whole blood.

$$C_{f,p} = \frac{C_{\text{blood}}}{(1-H)(1+K_{bf}) + HK_e} \quad [4]$$

Bupivacaine Dosage

Dosage of bupivacaine was modeled as a constant rate IV injection over a period of time on the order of minutes. Conservation of mass was confirmed by monitoring the cumulative dosage, clearance, and drug content in each organ and blood compartment.

Model Parameterization

The PBPK model contains no fitting parameters. Plasma protein binding parameters for bupivacaine were taken from Denson *et al.*¹⁴ Physiological parameters were chosen to be representative of a healthy adult male^{19,20} (body weight 72 kg). Plasma–tissue partition coefficients were taken from Howell *et al.*¹⁷ and are based on the mechanistic model of Rodgers *et al.*²¹ Hepatic elimination of bupivacaine is modeled using a constant intrinsic unbound clearance determined from literature values of the hepatic extraction ratio^{5,7,22} (further detail is given in the appendix). The intrinsic metabolic clearance of bupivacaine is assumed to be unaltered by the presence of lipid.

Model Validation

The model was validated by comparison with data obtained in studies of bupivacaine pharmacokinetics performed by Burm *et al.*¹⁰ and Tucker *et al.*^{6,9,11} These investigators studied the systemic distribution and elimination of bupivacaine in healthy individuals using limited doses administered intravenously. From plasma concentration curves, key pharmacokinetic quantities were evaluated, including (1) characteristic half-lives, (2) systemic clearance, and (3) volume of distribution at steady state, $V_{d,ss}$. These quantities, as well as a direct comparison of the plasma concentration–time curve, were used to validate the PBPK model.

Modeling Lipid Therapy

The validated model was used to investigate the potential impact of the hypothesized lipid sink mechanism. Following the clinical report of Marwick *et al.*,²³ bupivacaine dosage was modeled as an accidental IV injection of 112.5 mg over 3 min. Five minutes after the cessation of drug infusion, administration of a bolus of ILE was simulated. As per the existing guidelines for lipid therapy,[‡] the bolus was modeled as 1.5 ml/kg and was followed by a simulated infusion of 0.25 ml · kg⁻¹ · min⁻¹. The 90-s duration of the lipid bolus mimicked that reported by Marwick. After a 3-min interval, this was followed by a 60-min infusion.

The lipid emulsion is modeled after a 20% long chain triglyceride emulsion, with bupivacaine binding behavior based on the findings of Mazoit²⁴; *in vitro* measures of the concentration-dependent uptake of bupivacaine by one volume% lipid (20% emulsion diluted with buffer to a composition of one part soybean oil to 99 parts aqueous medium, *i.e.*, 1% by volume) yielded a binding capacity of 2130 μM and dissociation constant (K_d) of 665 μM for racemic bupivacaine at 37°C and pH 7.4. On this basis, the plasma–lipid partition coefficient for bupivacaine can be modeled as follows:

$$K_{bf} = \frac{C_{\text{lip},p}}{C_{f,p}} = \frac{B_{\max} \text{LIP}}{K_{d,\text{lip}} + C_{f,p}} \quad [5]$$

where $C_{\text{lip},p}$ is the concentration of bupivacaine bound to lipid in plasma. We have chosen here to reexpress the binding capacity, B_{\max} , as per unit volume of oil ($B_{\max} = 0.213$ M; derivation detailed in the appendix); LIP (the time-variant volume fraction of plasma lipid) then the time-dependent volume fraction of plasma lipid.

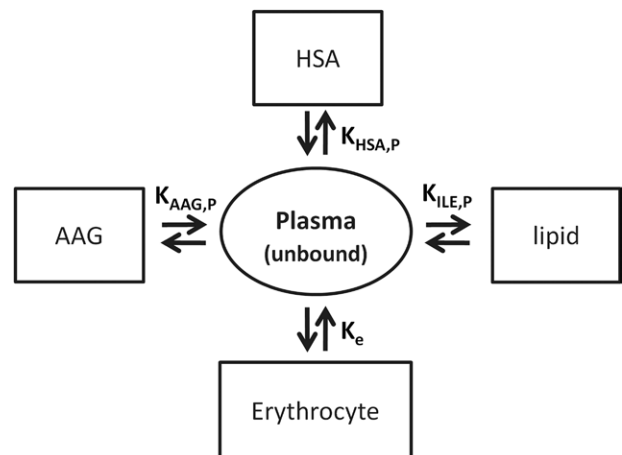


Fig. 2. Assumption regarding partitioning of bupivacaine between unbound, protein-bound, lipid, and erythrocyte populations in blood. Each binding agent is assumed to interact independently with the unbound drug in plasma. AAG = α -1 acid glycoprotein; HSA = human serum albumin; ILE = intravenous lipid emulsion; $K_{\text{AAG},P}$, $K_{\text{HSA},P}$ = protein–plasma partition coefficients; $K_{\text{ILE},P}$ = lipid–plasma partition coefficient; K_e = erythrocyte–plasma partition coefficient.

‡ Weinberg GL: LipidRescue: Resuscitation for cardiac toxicity. Available at: <http://lipidrescue.squarespace.com>. Accessed January 26, 2013.

Table 1. Bupivacaine Pharmacokinetics

	Bupivacaine Dosage	$t_{1/2,D}$ [min]	$t_{1/2,E}$ [min]	CL_s [l/min]	$V_{d,ss}$ [l]	MRT [min]	
PBPK simulated	29.2 mg, over 10 min	12.2	152	0.64	71	116	
Burm ¹⁰		15.3 ± 9.9	111 ± 32	0.61 ± 0.15	66 ± 23	—	
PBPK simulated		$t_{1/2,rapid}$	1.67	131	0.65	73	118
		$t_{1/2,inter}$	27.1				
Simulated using assumptions of Howell ¹⁷	44.2 mg, over 10 min	$t_{1/2,rapid}$	4.40	398	0.53	138	267
		$t_{1/2,inter}$	67.3				
Tucker ⁶		$t_{1/2,rapid}$	2.7 ± 1.8	162 ± 78	0.58 ± 0.23	73 ± 26	—
		$t_{1/2,inter}$	28.8 ± 11.4				

Pharmacokinetic quantities drawn from the literature are compared with those derived from the PBPK model. For each comparison, the approach used in estimating pharmacokinetic quantities is the same as that used in the corresponding clinical study.

CL_s = systemic clearance; MRT = mean residence time; PBPK = physiologically based pharmacokinetic; $t_{1/2,D}$ = distribution half-life; $t_{1/2,E}$ = elimination half-life; $t_{1/2,rapid}$ and $t_{1/2,inter}$ = rapid and intermediate half-lives, respectively, for the three exponential model employed by Tucker⁶; $V_{d,ss}$ = steady-state volume of distribution.

Lipid-bound bupivacaine is assumed to be in instantaneous equilibrium with unbound bupivacaine in the aqueous plasma. Anesthetic in the blood stream is thus taken to be distributed between plasma macromolecules, erythrocytes, lipid, and the aqueous plasma—with the distribution governed by the independent equilibrium partitioning relationships with unbound bupivacaine in the plasma (fig. 2). Hence the relationship between whole blood and plasma unbound concentrations remains as described in Equation [4], with K_{bf} augmented by the lipid:anesthetic binding coefficient (Equation [6]).

$$K_{bf} = \frac{C_{b,p}}{C_{f,p}} = \frac{B_{max,LIP}}{K_{d,lip} + C_{f,p}} + \sum_j \frac{[np]_j}{[K]_j + [C_{f,p}]_j} \quad [6]$$

To investigate the validity of this scheme, which employs lipid-binding parameters quantified in a buffer, it is desirable to test the predictive quality of the ILE-binding parameters by comparison with experimental measures of bupivacaine uptake from plasma.

In Weinberg's 1998 publication²⁵ that first reported the ability of IV lipid to mitigate the toxic effects of bupivacaine, lipid:aqueous partitioning was quantified in rat plasma mixed with an equal volume of a 30% lipid emulsion and spiked with 93 µg/ml (323 µM) anesthetic—yielding a system of 15 parts oil per 100 ml volume, *i.e.*, 15 volume%. Estimates of protein-binding parameters for rat plasma were obtained from Coyle *et al.*,¹⁶ and our equilibrium partitioning scheme was used to predict lipid uptake of bupivacaine. The predicted 79% uptake by 15 volume% lipid in plasma agrees well with Weinberg's measurement of 75.3 ± 1.32%. Similar agreement is seen for the case of 2% lipid in human serum. Ruan *et al.*²⁶ report the uptake of 22.3% of total bupivacaine from human serum containing 10 µg/ml (34.7 µM) of anesthetic.

Our model yields a predicted fractional uptake of 20%. As the modeled uptake agrees reasonably well with experimental observations, the parameters obtained from Mazoit's work were deemed appropriate for use in the PBPK model. Possible sources of discrepancy include the assumption of linearity in the lipid-binding capacity as a function of lipid volume fraction and interindividual variations in plasma protein binding.

Lipid Distribution

In vitro experiments have demonstrated that the bulk of bupivacaine uptake by lipid emulsions occurs within 1 min of mixing²⁴—a time similar to that required for lipid to be distributed throughout the bloodstream. Furthermore, ILE droplets have

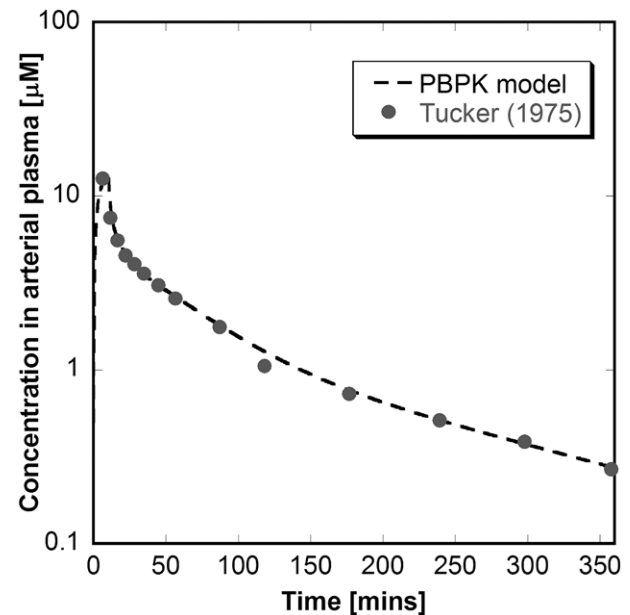


Fig. 3. Arterial plasma concentration following intravenous delivery of 66.7 mg bupivacaine. The PBPK-predicted curve is plotted alongside the observations of Tucker.¹¹ PBPK = physiologically based pharmacokinetic.

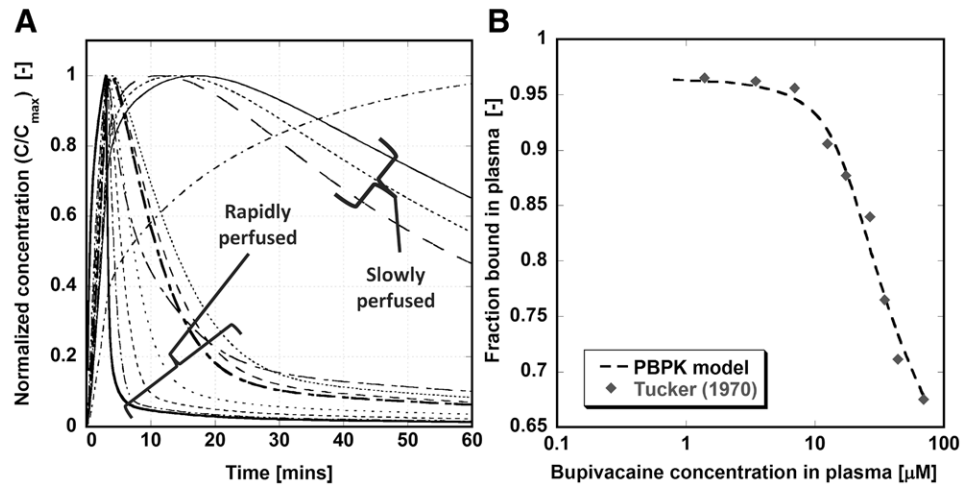


Fig. 4. An intravenous dose of 112.5 mg of bupivacaine administered over 3 min. (A) Concentration–time curves for the 12 organs included in the physiologically based pharmacokinetic (PBPK) model. Concentration for each tissue, C , is normalized against the maximum concentration occurring in that tissue, C_{\max} . (B) Fraction of bupivacaine protein-bound in plasma as a function of total plasma concentration. Data from Tucker.⁹

been observed to have a volume of distribution indistinguishable from plasma volume.^{27,28} Thus, an assumption of rapid lipid distribution about the body and rapid equilibration in the blood compartment is likely justified. Lipid is assumed to be confined to the capillary bed upon passage through organs.

Results

Model Validation

Bupivacaine administration was modeled as a 10-min IV infusion of (1) 29.2 mg, (2) 44.2 mg, or (3) 66.7 mg, as appropriate to the three studies^{6,10,11} used in model validation. Plasma concentration curves were used to evaluate the pharmacokinetic quantities of interest (table 1). In case (1), plasma concentration data were fitted by the same biexponential model used by Burm *et al.*¹⁰ A weighted nonlinear least squares regression was performed to obtain characteristic half-lives (distribution, $t_{1/2,D}$, and elimination, $t_{1/2,E}$). The simulation results yielded half-lives of 12 and 152 min for distribution and elimination, respectively. Following the approach of Tucker *et al.*,⁶ in case (2), characteristic half-lives were evaluated by fitting a three-exponential model yielding rapid, intermediate, and elimination half-lives ($t_{1/2, \text{rapid}}$, $t_{1/2, \text{inter}}$, and $t_{1/2,E}$). A final comparison was made by superimposing the plasma concentration curve from the PBPK model with data obtained in a third study (fig. 3).¹¹ Also shown in table 1 are the pharmacokinetic quantities predicted by implementing the assumptions of a similar PBPK model reported by Howell *et al.*,¹⁷ which differs from the current model in certain key respects.

§ Data extracted from Tucker *et al.*¹¹ using WebPlotDigitizer. Available at: <http://arohatgi.info/WebPlotDigitizer/>. Accessed January 26, 2013.

Accidental IV Administration of 112.5 mg

Following a simulated overdose of bupivacaine, a rapid increase in bupivacaine content occurs for rapidly perfused organs. Concentration–time curves (fig. 4A) display maxima at—or shortly following—the end of the bupivacaine infusion for all rapidly perfused organs. A lag of ≈ 10 –20 min is observed for more slowly perfused organs (bone, muscle, and skin). The maximum for adipose tissue occurs at ≈ 1.5 h. The fraction of bupivacaine bound in the blood stream decreases as the anesthetic concentration increases (fig. 4B). The fraction unbound in plasma increases from $\approx 3.5\%$ to a maximum of $\approx 27\%$ ($C_{f,p} = 11.8 \mu\text{M}$, $C_{\text{blood}} = 31.7 \mu\text{M}$) at the end of the bupivacaine infusion.

ILE Therapy

Upon administration of lipid therapy, there appears to be little change in the shape of the normalized concentration curves for the organs in the PBPK model (fig. 5A). However, there is a more rapid decrease in concentration for those organs in the distribution phase when lipid administration begins. The maximum bupivacaine concentration in each organ is essentially unchanged (data not shown), with the exception of the adipose tissue, for which there is a small increase of 3%. The time to maximum bupivacaine concentration in adipose tissue is reduced by 4%.

The impact of the lipid sink is more clearly observed when tissue concentration is expressed as a function of what would occur in the absence of lipid therapy. Figure 5B demonstrates the reduction in concentration that occurs due to lipid binding of bupivacaine. Within the first 3 min of ILE therapy, the concentration of bupivacaine in heart tissue is reduced by 11%. Within the first 15 min, brain tissue content is reduced by 18%. The slowly perfused adipose tissue exhibits a modest increase in tissue concentration in the presence of lipid. Figure 6 shows the

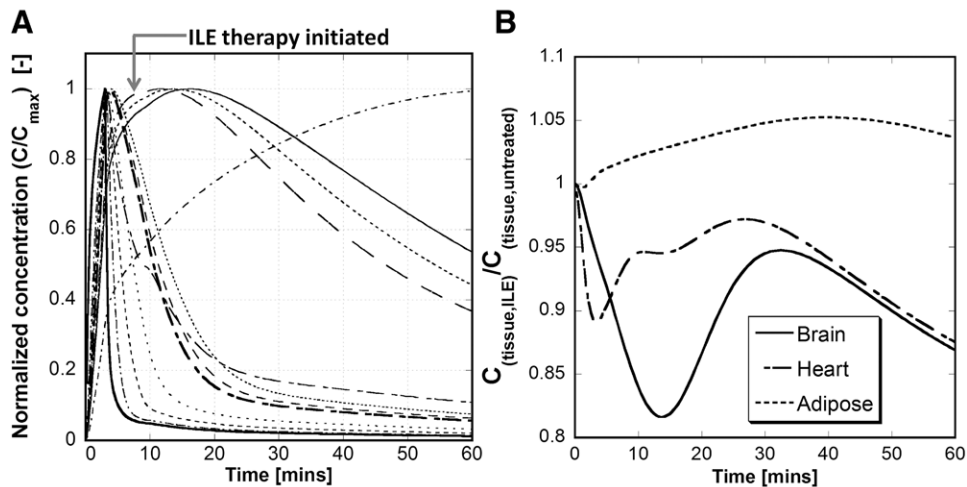


Fig. 5. An intravenous dose of 112.5 mg of bupivacaine administered over 3 min followed by simulated lipid emulsion therapy (bolus: 1.5 ml/kg over 1.5 min; continuous infusion: 0.25 ml · kg⁻¹ · min⁻¹ over 60 min). (A) Normalized concentration–time curves for the 12 organs included in the physiologically based pharmacokinetic model. Abscissa indicates time following the initiation of the bupivacaine dose. (B) Bupivacaine concentration curves in heart, brain, and adipose tissues. Concentrations ($C_{\text{tissue,ILE}}$) are expressed relative to those observed for the same anesthetic dose in the absence of lipid ($C_{\text{tissue,untreated}}$). Abscissa indicates time following the initiation of lipid therapy. C = tissue concentration; C_{max} = maximum concentration in tissue; ILE = intravenous lipid emulsion.

maximal extent to which tissue concentration is altered (relative to the untreated case) in each of the 12 PBPK organs within the first 15 min of lipid therapy. Evaluation of the area under the concentration curve ($AUC_{0-\infty}$) for each organ tissue (table 2) reveals a decrease of up to 12% relative to the untreated case. The exception is the

liver, where bupivacaine concentration is elevated during lipid administration and subsequently reduced relative to the case of untreated overdose; in liver tissue, $AUC_{0-\infty}$ is unchanged. The systemic clearance, volume of distribution, and mean residence time for bupivacaine are reduced by 8, 17, and 9%, respectively.

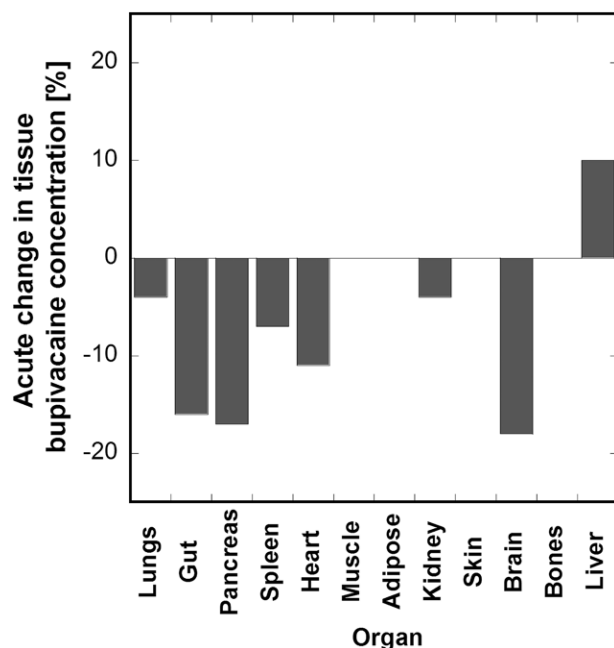


Fig. 6. The impact of simulated intravenous lipid emulsion therapy on bupivacaine concentration in tissues during the first 15 min of lipid administration. The ordinate indicates fractional reduction in bupivacaine concentration relative to the case of untreated overdose.

Blood Concentration

The effluent blood from the brain and heart exhibits an increase in bupivacaine concentration upon lipid administration (fig. 7). The effect is more pronounced in the case of the brain, where a clear secondary maximum is observed. For both organs, the effluent blood concentration after lipid infusion ends ($t = 73$ min) is reduced compared to the case of untreated overdose. Figure 8 represents plasma concentrations in the arterial blood (total and unbound) normalized by that which would be observed in the absence of lipid therapy. The total plasma concentration is elevated in the presence of lipid. In contrast, the unbound concentration is reduced.

Lipid–bupivacaine Binding Efficacy

If the lipid sink is the dominating mechanism underlying the success of lipid resuscitation, the efficacy of the therapy should improve with (1) increased quantity of lipid in the blood stream, which would increase the effective lipid-binding capacity; or (2) increased ILE–bupivacaine binding affinity (inverse of dissociation constant, K_d), which implies modifying the emulsion formulation in some as yet poorly understood way. As there are potentially negative physiological implications associated with increasing lipid dosage, further PBPK simulations focused on hypothetical

Table 2. AUC_{tissue} Evaluated for a 112.5-mg Dose of Bupivacaine Administered over 3 min

Compartment	AUC_{tissue} ($\mu\text{M}\cdot\text{min}$)		AUC Reduction due to Therapy	Acute Reduction in Tissue Concentration of Bupivacaine (Time following Initiation of Therapy)
	Untreated	Intralipid Therapy		
Blood (arterial)	559	608	—	—
Lungs	559	609	4%	4% (14 min)
Gut	1,074	998	7%	16% (10 min)
Pancreas	1,111	1,027	8%	17% (11 min)
Spleen	430	413	4%	7% (2 min)
Heart	546	518	5%	11% (3 min)
Muscle	313	277	11%	—
Adipose	1,438	1,392	3%	—
Kidney	695	670	4%	4% (13 min)
Skin	1,181	1,044	12%	—
Brain	969	892	8%	18% (15 min)
Bones	358	316	12%	—
Liver	275	275	0%	—

Values are provided for the simulated IV dose with and without lipid therapy. Also shown is the acute reduction in tissue concentration within the first 15 min of lipid therapy and the time at which this is observed.

AUC_{tissue} = area under the tissue concentration-time curve evaluated from time zero and extrapolated to infinity.

lipid emulsions with altered binding affinity. Simulation of ILE therapy was repeated for values of the lipid-binding affinity increased by factors of 2, 4, and 8 ($K_a = 1,504, 3,008, 6,015, 12,030 \text{ M}^{-1}$, respectively). The corresponding acute reduction in bupivacaine concentration in the heart and brain is shown in figure 9. A doubling of the binding affinity yields an 18 and 29% reduction in bupivacaine concentration in brain and heart tissues, respectively, within the first 15 min of ILE administration. The dependence of this reduction on the lipid-binding affinity is logarithmic, such that an increase in binding affinity by a factor of 8 yields a reduction of 40 and 51% for heart and brain tissues, respectively. The drop in tissue concentration also occurs more rapidly as the binding affinity is increased (data not shown).

Discussion

Model Validity

The secondary pharmacokinetic quantities yielded by our model are in excellent agreement with the clinical observations of Burm *et al.*¹⁰ and Tucker.^{6,11} Very good agreement is also observed for the plasma concentration curve. Quantitative agreement is observed for the trend in plasma binding as a function of bupivacaine concentration. This is in contrast to the results obtained upon implementing the assumptions of Howell *et al.*¹⁷, who reported a PBPK study of liposome-mediated toxicity reversal. A principal difference between their work and ours is the handling of protein binding. In their model, protein binding was treated as single site and concentration independent, rather than the explicit modeling

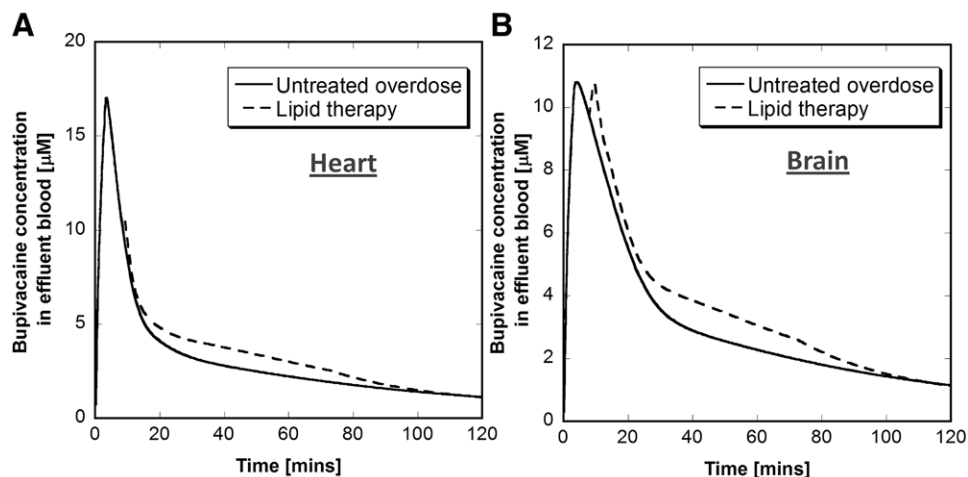


Fig. 7. Bupivacaine concentration in effluent blood for the (A) heart and (B) brain. Introduction of lipid (at $t = 8$ min) causes an increase in blood content of bupivacaine.

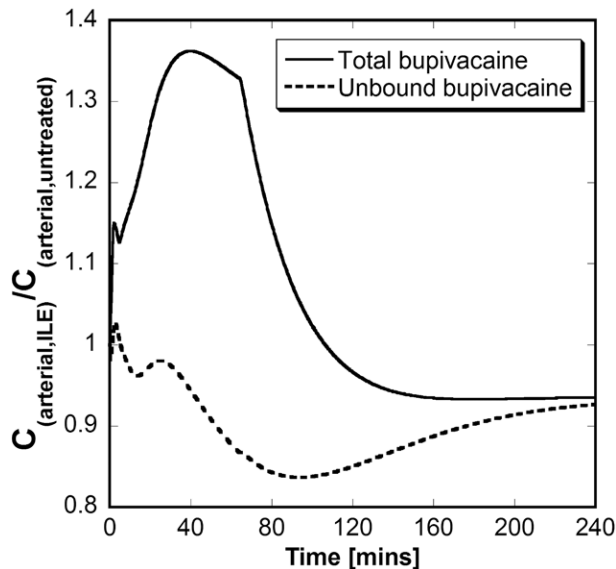


Fig. 8. Bupivacaine concentration curves in arterial blood as a consequence of lipid therapy. Concentrations are expressed relative to those observed for the same anesthetic dose in the absence of lipid. Abscissa indicates time following the initiation of lipid therapy. Total concentration in arterial blood is increased upon lipid administration. Unbound concentration in arterial blood is reduced in the presence of lipid.

of two distinct binding sites (α -1 acid glycoprotein and human serum albumin) that is employed here. While the protein-bound fraction of bupivacaine can be approximated as a constant for low blood concentrations, binding becomes nonlinear thereafter.⁹ For the low doses of bupivacaine employed in our model validation, the fixed protein binding of 90% used by Howell *et al.* allows for a larger unbound concentration in the plasma, and hence a greater partitioning of drug into organ tissues than in our model, which predicts protein uptake of $\approx 97\%$. The two models also differ in the value of the erythrocyte partition coefficient and the handling of organ mass balances. No distinction between tissue and organ concentration is made in the prior model; each organ is treated as well-stirred and effluent blood is taken to be at equilibrium with the organ concentration. This leads to an inconsistent mass balance, as detailed by Berezhkovskiy.²⁹ When incorporated into our model, the approximations detailed by Howell *et al.*¹⁷ lead to an overestimation of the volume of distribution and characteristic bupivacaine half-lives (see the appendix).

Predicted Effect of the Lipid Sink

The results suggest that a lipid sink mechanism would result in a reduction of the unbound concentration of bupivacaine in plasma, accompanied by a redistribution of anesthetic from organ tissues to the blood stream. The presence of lipid shifts tissue–blood partition coefficients in such a way as to increase the concentration of

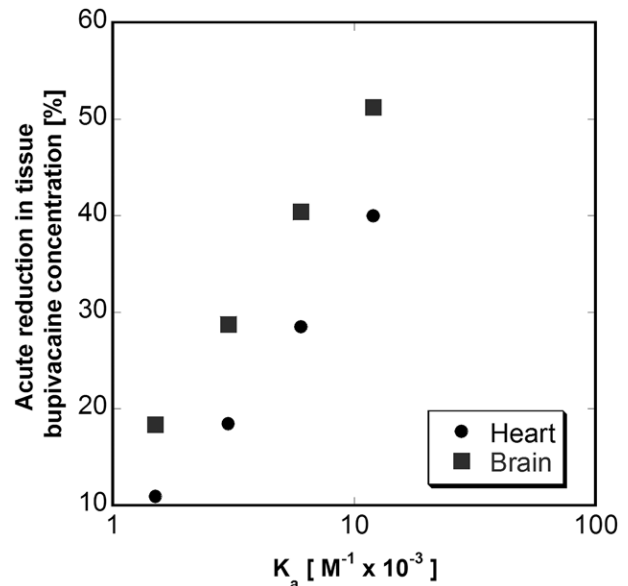


Fig. 9. Effect of increased intravenous lipid emulsion binding affinity. The acute reduction in tissue bupivacaine increases logarithmically as a function of lipid–bupivacaine binding affinity.

bupivacaine in blood and thereby increase the outflow of anesthetic from organs. In the case of the heart, this results in a “bump” (region of elevated concentration) in the concentration curve, similar to that observed by Weinberg *et al.*³⁰ in a study of accelerated efflux from isolated heart models. The reduction in time to maximum bupivacaine concentration observed for slowly perfused organs suggests that lipid should transiently accelerate the distribution of bupivacaine to poorly perfused tissues. The timescale on which bupivacaine in tissues is reduced due to lipid administration varies from organ to organ, with the concentration in the heart falling within minutes (*i.e.*, during the lipid bolus). The extent to which heart concentration is reduced is modest ($\approx 11\%$). The concentration of bupivacaine in brain tissue is reduced by a larger extent (18%), but over a longer time frame (≈ 15 min). As the effects of lipid infusions tend to be observed within a few minutes,¹ the extent to which washout from organs is increased by the lipid sink may not be adequate—in isolation—to explain lipid resuscitation. However, hemodynamic improvements resulting from hypothesized metabolic or inotropic effects may couple with the sink mechanism to yield more rapid bupivacaine washout.

Bupivacaine concentration in liver tissue is predicted to be elevated during lipid administration. The corresponding increase in the concentration unbound in liver blood leads to an increase in the rate of bupivacaine metabolism by up to 13%. This occurs despite the reduction in hepatic extraction that results when the presence of lipid reduces the free fraction of anesthetic in liver blood. The decrease in free fraction of bupivacaine coincides with an increase in whole blood concentration such that the unbound concentration

of bupivacaine and the rate of anesthetic metabolism are, in fact, elevated.

Increasing the binding affinity of ILE for the toxin in question would make the lipid sink a more viable mechanism. Given the large dissociation constant for bupivacaine binding by lipid (large relative to typical unbound physiological concentrations of the anesthetic, *i.e.*, $K_d \gg C_{f,p}$), a multiplicative increase in the lipid–bupivacaine binding affinity, $K_{a,lip}$, is indistinguishable from the same increase in the effective lipid-binding capacity, $B_{max} LIP$; *viz* Equation [7]. Thus altering the method of lipid administration to increase the lipid volume fraction (within safe limits) would be expected to improve the therapeutic benefit of existing ILE formulations.

$$K_{bf} = \frac{B_{max} LIP}{K_{d,lip} + C_{f,p}} \approx B_{max} K_{a,lip} LIP \quad [7]$$

Extrapolating from In Vitro Measures of Uptake

The capacity of lipid to uptake bupivacaine is frequently measured at high lipid concentration,²⁵ with high bupivacaine concentration (up to $\approx 1,000 \mu M$),^{24,25,31–33} or in the absence of plasma macromolecules.²⁴ In these cases, a misleadingly high level of drug uptake is observed. However, even at moderate concentration, lipid exhibits a large uptake capability in the absence of plasma proteins (*e.g.*, in buffer).²⁴ When lipid is introduced into the bloodstream, it competes with erythrocytes and plasma proteins for binding of the anesthetic. In a buffer, 2 volume% lipid (oil droplets) is expected to bind $\approx 90\%$ of bupivacaine. In whole blood, predicted uptake drops to $\approx 50\%$ —which is still encouraging. Stehr *et al.*³³ observed this effect of competitive binding in a series of *in vitro* experiments where a lipid emulsion (Structolipid, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) was observed to uptake L-bupivacaine more readily from a buffer than from human plasma. Unfortunately, a substantial fraction of anesthetic bound to lipid in blood does not imply an equivalent increase in the overall bound fraction of bupivacaine when compared to lipid-free blood. Rather, bupivacaine is redistributed among the available binding agents; at high anesthetic concentrations, this principally involves serum albumin and lipid, as the glycoprotein population is saturated at concentrations $> 30 \mu M$. The redistribution of bupivacaine observed in our model is consistent with modest increases in bupivacaine uptake observed *in vitro* by researchers studying lipid–bupivacaine interactions at physiologically relevant concentrations in serum.^{26,34,35}

Limitations

The PBPK results should be interpreted with caution. Validation of the model and observations in the literature suggest that the assumptions of perfusion limitation and rapid equilibria are appropriate. However, assumptions

made regarding erythrocyte binding, lipid distribution, and the fate of anesthetic released from metabolized droplets may require further scrutiny. The model does not address interindividual variation in drug-specific and physiological parameters. However, results produced using alternative measures of plasma protein-binding capacity and affinity have yielded qualitative agreement with the results presented here. The distribution of lipid from the venous compartment, where it is administered, to the rest of the body has not been explicitly modeled. In addition, pharmacodynamic effects have been ignored. Hence variations in cardiac output and its implications for bupivacaine clearance^{7,36–38} have not been addressed. Likewise, we assume bupivacaine metabolism to be unsaturated in the range of concentrations relevant to this study. Also neglected are pH-dependent variations in lipid or protein binding. Cardiac arrest may be swiftly followed by acidosis, and protein binding has been observed to be sensitive to pH.¹⁶ A drop in pH to 7.0 tends to reduce protein binding of bupivacaine¹⁶; the resulting increase in free bupivacaine may allow the lipid scavenger to play a more significant role in the uptake of bupivacaine. The influence of pH on the binding action of the lipid is not yet well understood, with some researchers finding lipid uptake to be pH independent (in serum),³⁵ while others have observed pH sensitivity (in buffer).^{24,26} Hemodynamic effects, pH effects, hemodilution, and lipid pharmacokinetics will be considered in future implementations of the model.

Appendix: Governing Equations of the Physiologically Based Pharmacokinetic Model

Mass Balances

Noneliminating Organs (Except Lungs). For noneliminating organs, the mass balance describing the rate of bupivacaine accumulation is given by Equation 1:

$$V_{org} \frac{dC_{org}}{dt} = Q_{org} \left(C_{artery} - \frac{C_{tis}}{R_{tb,org}} \right) \quad [1]$$

where V_{org} is the total organ volume, C_{tis} is the bupivacaine concentration in organ tissue, Q_{org} is the rate of blood supply to the organ, C_{artery} is the total bupivacaine concentration in the arterial blood, and R_{tb} is a tissue–blood partition coefficient describing the equilibrium relationship between C_{tis} and the bupivacaine concentration in the blood leaving the organ (*i.e.*, perfusion limited transport is assumed). C_{org} is the volume weighted concentration of bupivacaine in the organ, with contributions from the blood stream (concentration $C_{blood,eq} = C_{tis}/R_{tb}$) and the tissue as follows²⁹:

$$C_{org} = C_{tis} (1 - f_{vasc}) + C_{blood,eq} f_{vasc} \quad [2]$$

where f_{vasc} is the vascular fraction of the organ volume.

Lungs. The lung mass balance differs in that the feed of blood originates from the venous compartment:

$$V_{lung} \frac{dC_{lung}}{dt} = Q_{lung} \left(C_{venous} - \frac{C_{tis, lung}}{R_{tb, lung}} \right) \quad [3]$$

Liver. Elimination is modeled using intrinsic hepatic clearance as per Equation [4],

$$V_{liver} \frac{dC_{liver}}{dt} = Q_{hepatic} C_{artery} + \sum_i Q_i \frac{C_{tis, i}}{R_{tb, i}} - Q_{liver} \left(\frac{C_{tis, liver}}{R_{tb, liver}} \right) - Clu_{int} Cu_b \quad [4]$$

where i = gut, spleen, or pancreas; Clu_{int} is the intrinsic unbound hepatic clearance; and Cu_b is the unbound concentration of bupivacaine in liver blood. We assume that hepatic flow is constant and metabolism exhibits unsaturated kinetics. The intrinsic unbound clearance is treated as a constant and is obtained from equation [5], which corresponds to the well-stirred model of hepatic clearance.²²

$$Clu_{int} = \frac{EQ}{(1-E) f_{u, b}} \quad [5]$$

Here, Q is the total liver blood flow (1.66 l/min), E is the extraction ratio ($E = 0.37$),^{5,7} and $f_{u, b}$ is the fraction of bupivacaine unbound in liver blood. For therapeutic blood levels of bupivacaine, the PBPk protein-binding model predicts $f_{u, b} = 0.033$, giving $Clu_{int} = 29.2$ l/min.

Bupivacaine has been observed to alter hepatic flow, particular after lengthy periods of infusion. An augmentation of hepatic flow would increase the rate of bupivacaine clearance.^{5,36–38} For the purposes of this model, which relates to a bolus infusion of bupivacaine, we ignore any such effect.

Blood Compartments. Mass balances for the venous and arterial compartments allow for inflow from all organs excluding the lung, digestive organs, pancreas, and spleen and outflow to all organs excluding the lung, respectively.

$$V_{vein} \frac{dC_{vein}}{dt} = \sum_{supplying\ organs} Q_i \frac{C_{tis, i}}{R_{tb, i}} - Q_{co} C_{vein} \quad [6]$$

$$V_{artery} \frac{dC_{artery}}{dt} = Q_{co} \frac{C_{tis, lung}}{R_{tb, lung}} - Q_{co} C_{artery} \quad [7]$$

where Q_{co} is the total cardiac output.

Tissue–Blood Partitioning. The R_{tb} parameter in the mass balances is calculated by assuming an equilibrium partitioning between the organ tissue and blood subcompartments. It

can be shown that the tissue–plasma and tissue–whole blood partition coefficients are related by

$$R_{tb} = \frac{R_p}{(1-H)(1+K_{bf}) + HK_e}$$

where H is the hematocrit, and the factors of $1-H$ and H in the denominator are included to correct for the difference in volume between the whole blood, plasma, and hematocrit.

Bupivacaine Dosage. During dosage periods, the mass balance on the vein compartment includes an additional input term representing this constant rate infusion:

$$V_{vein} \frac{dC_{vein}}{dt} = \sum_{supplying\ organs} Q_i \frac{C_{tis, i}}{R_{tb, i}} - Q_{co} C_{vein} + \frac{m_{dosage}}{\tau_{dosage}} \quad [9]$$

where m_{dosage} and τ_{dosage} are the bupivacaine dosage and infusion duration, respectively.

Lipid Balance. The lipid balance includes relevant terms for lipid administration and lipid metabolism.

$$\frac{dV_{lip}}{dt} = kV_{lip} + Q$$

where V_{lip} is the volume of plasma lipid at time t , k is the first-order rate constant for lipid elimination, and Q is the rate of lipid infusion.

The binding capacity quantified by Mazoit *et al.*²⁴ is appropriate to a system containing 1 part lipid (oil) per 100 parts of total volume. As the lipid volume fraction in plasma changes over the course of the PBPk simulation—due to emulsion administration and lipid metabolism—it is desirable to reexpress the binding capacity such that it remains a time independent constant as follows:

$$\begin{aligned} & 2130 \frac{\mu\text{moles bupivacaine}}{1 \text{ L of vol \% emulsion}} \times \frac{1 \text{ L of vol \% emulsion}}{0.01 \text{ L of lipid}} \\ & = 213,000 \frac{\mu\text{moles bupivacaine}}{1 \text{ L of lipid}} \text{ or } 0.213 \text{ M} \end{aligned} \quad [11]$$

Here, we have converted the volume basis of the binding capacity to be the actual volume of lipid and not the volume of emulsion. By employing this representation of the binding capacity, the time-dependent character of the lipid–bupivacaine partition coefficient is described solely by the time-variant volume fraction of plasma lipid, $LIP(t)$, *viz* equation [12].

$$K_{bf} = \frac{B_{max} LIP(t)}{K_{d, lip} + C_{f, p}}; LIP(t) = \frac{V_{oil}(t)}{V_{plasma}} \quad [12]$$

Note that the altered basis for B_{max} implies the same change in basis for LIP . LIP here is the volume fraction of lipid (oil)—not the volume fraction of emulsion, as defined by Mazoit.

Adopting the Approximations of Howell *et al.* In adopting the approach of Howell *et al.*,¹⁷ we employ a concentration independent, single-site model for protein binding with partition coefficient $K = 9.0$ representing the ratio between protein-bound bupivacaine and free bupivacaine in plasma. We also adopt a partition coefficient $K = 1.64$ dictating the ratio between erythrocyte-associated bupivacaine and free bupivacaine in plasma. Bound and free concentrations are defined based on whole blood volume so as to remain consistent with the work of Howell. The relationship between R_{tp} and R_{tb} is altered accordingly. Finally, we alter the organ mass balances in our model to remove the distinction between tissue and organ concentrations such that the governing equations become

$$V_{org} \frac{dC_{org}}{dt} = Q_{org} \left(C_{artery} - \frac{C_{org}}{R_{tb,org}} \right) \quad [13]$$

$$V_{liver} \frac{dc_{liver}}{dt} = Q_{hepatic} C_{artery} + \sum_i Q_i \frac{C_{org,i}}{R_{tb,i}} - Q_{liver} \left(\frac{C_{liver}}{R_{tb,liver}} \right) - Cl_{int} C_u \quad [14]$$

The tissue area under the concentration curve values resulting from the current approach and the prior model are compared in table 3.

Table 3. Comparing Two Approaches to Modeling Bupivacaine Pharmacokinetics

Compartment	AUC _{tissue} , $\mu\text{M}\cdot\text{min}$	
	Current Model	Prior Model
Blood (arterial)	237	291
Lungs	224	592
Gut	328	878
Pancreas	343	924
Spleen	117	310
Heart	156	414
Muscle	113	318
Adipose	574	1646
Kidney	181	478
Skin	430	1221
Brain	305	824
Bones	132	377
Liver	108	358
CL	0.65	0.53
$V_{d,ss}$	73	138
MRT	118	267

Results from the present model are compared with an alternative approach employing the assumptions of a previously reported pharmacokinetic model for bupivacaine.¹⁷

AUC_{tissue} = area under the tissue concentration-time curve evaluated from time zero and extrapolated to infinity; CL_s = systemic clearance; MRT = mean residence time; $V_{d,ss}$ = steady-state volume of distribution.

References

- Cave G, Harvey M: Intravenous lipid emulsion as antidote beyond local anesthetic toxicity: A systematic review. *Acad Emerg Med* 2009; 16:815–24
- Bern S, Akpa BS, Kuo I, Weinberg G: Lipid resuscitation: A life-saving antidote for local anesthetic toxicity. *Curr Pharm Biotechnol* 2011; 12:313–9
- Toledo P: The role of lipid emulsion during advanced cardiac life support for local anesthetic toxicity. *Int J Obstet Anesth* 2011; 20:60–3
- Weinberg GL: Lipid emulsion infusion: Resuscitation for local anesthetic and other drug overdose. *ANESTHESIOLOGY* 2012; 117:180–7
- Veering BT, Burm AGL: Pharmacokinetics and pharmacodynamics of medullar agents: A). Local anaesthetics. In *new developments in epidural and spinal drugs administration*. Balliere Clin Anaesthesiol 1993; 7:557–77
- Tucker GT, Mather LE: Clinical pharmacokinetics of local anaesthetics. *Clin Pharmacokinet* 1979; 4:241–78
- Tucker GT, Wiklund L, Berlin-Wahlen A, Mather LE: Hepatic clearance of local anesthetics in man. *J Pharmacokinet Biopharm* 1977; 5:111–22
- Wood M: Plasma drug binding: Implications for anesthesiologists. *Anesth Analg* 1986; 65:786–804
- Tucker GT, Boyes RN, Bridenbaugh PO, Moore DC: Binding of anilide-type local anesthetics in human plasma. I. Relationships between binding, physicochemical properties, and anesthetic activity. *ANESTHESIOLOGY* 1970; 33:287–303
- Burm AG, de Boer AG, van Kleef JW, Vermeulen NP, de Leede LG, Spierdijk J, Breimer DD: Pharmacokinetics of lidocaine and bupivacaine and stable isotope labelled analogues: A study in healthy volunteers. *Biopharm Drug Dispos* 1988; 9:85–95
- Tucker GT, Mather LE: Pharmacology of local anaesthetic agents. *Pharmacokinetics of local anaesthetic agents*. *Br J Anaesth* 1975; 47:213–24
- Veering BT, Burm AG, Gladines MPRR, Spierdijk J: Age does not influence the serum protein binding of bupivacaine. *Br J Clin Pharmacol* 1991; 32:501–3
- Cousins MJ, Mather LE: Clinical pharmacology of local anesthetics. *Anaesth Intensive Care* 1980; 8:257–77
- Denson D, Coyle D, Thompson G, Myers J: Alpha 1-acid glycoprotein and albumin in human serum bupivacaine binding. *Clin Pharmacol Ther* 1984; 35:409–15
- Mazoit JX, Cao LS, Samii K: Binding of bupivacaine to human serum proteins, isolated albumin and isolated alpha-1-acid glycoprotein. Differences between the two enantiomers are partly due to cooperativity. *J Pharmacol Exp Ther* 1996; 276:109–15
- Coyle DE, Denson DD, Thompson GA, Myers JA, Arthur GR, Bridenbaugh PO: The influence of lactic acid on the serum protein binding of bupivacaine: Species differences. *ANESTHESIOLOGY* 1984; 61:127–33
- Howell BA, Chauhan A: A physiologically based pharmacokinetic (PBPK) model for predicting the efficacy of drug overdose treatment with liposomes in man. *J Pharm Sci* 2010; 99:3601–19
- Hinderling PH: Red blood cells: A neglected compartment in pharmacokinetics and pharmacodynamics. *Pharmacol Rev* 1997; 49:279–95
- Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP: Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol Ind Health* 1997; 13:407–84
- Edginton AN, Schmitt W, Willmann S: Development and evaluation of a generic physiologically based pharmacokinetic model for children. *Clin Pharmacokinet* 2006; 45:1013–34
- Rodgers T, Leahy D, Rowland M: Physiologically based pharmacokinetic modeling 1: Predicting the tissue distribution of moderate-to-strong bases. *J Pharm Sci* 2005; 94:1259–76

22. Wilkinson GR, Shand DG: Commentary: A physiological approach to hepatic drug clearance. *Clin Pharmacol Ther* 1975; 18:377–90
23. Marwick PC, Levin AI, Coetzee AR: Recurrence of cardiotoxicity after lipid rescue from bupivacaine-induced cardiac arrest. *Anesth Analg* 2009; 108:1344–6
24. Mazoit JX, Le Guen R, Bloeil H, Benhamou D: Binding of long-lasting local anesthetics to lipid emulsions. *ANESTHESIOLOGY* 2009; 110:380–6
25. Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ: Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. *ANESTHESIOLOGY* 1998; 88:1071–5
26. Ruan W, French D, Wong A, Drasner K, Wu AH: A mixed (long- and medium-chain) triglyceride lipid emulsion extracts local anesthetic from human serum *in vitro* more effectively than a long-chain emulsion. *ANESTHESIOLOGY* 2012; 116:334–9
27. Sakaeda T, Hirano K: Effect of composition on biological fate of oil particles after intravenous injection of O/W lipid emulsions. *J Drug Target* 1998; 6:273–84
28. Park Y, Damron BD, Miles JM, Harris WS: Measurement of human chylomicron triglyceride clearance with a labeled commercial lipid emulsion. *Lipids* 2001; 36:115–20
29. Berezhkovskiy LM: A valid equation for the well-stirred perfusion limited physiologically based pharmacokinetic model that consistently accounts for the blood-tissue drug distribution in the organ and the corresponding valid equation for the steady state volume of distribution. *J Pharm Sci* 2010; 99:475–85
30. Weinberg GL, Ripper R, Murphy P, Edelman LB, Hoffman W, Strichartz G, Feinstein DL: Lipid infusion accelerates removal of bupivacaine and recovery from bupivacaine toxicity in the isolated rat heart. *Reg Anesth Pain Med* 2006; 31:296–303
31. Martínez-Gómez MA, Villanueva-Camañas RM, Sagrado S, Medina-Hernández MJ: Evaluation of enantioselective binding of basic drugs to plasma by ACE. *Electrophoresis* 2007; 28:3056–63
32. Howell BA, Chauhan A: Bupivacaine binding to pegylated liposomes. *Anesth Analg* 2009; 109:678–82
33. Stehr SN, Ziegeler JC, Pexa A, Oertel R, Deussen A, Koch T, Hübner M: The effects of lipid infusion on myocardial function and bioenergetics in l-bupivacaine toxicity in the isolated rat heart. *Anesth Analg* 2007; 104:186–92
34. French D, Smollin C, Ruan W, Wong A, Drasner K, Wu AH: Partition constant and volume of distribution as predictors of clinical efficacy of lipid rescue for toxicological emergencies. *Clin Toxicol (Phila)* 2011; 49:801–9
35. Laine J, Lokajová J, Parshintsev J, Holopainen JM, Wiedmer SK: Interaction of a commercial lipid dispersion and local anesthetics in human plasma: Implications for drug trapping by “lipid-sinks”. *Anal Bioanal Chem* 2010; 396:2599–607
36. Wiklund L, Berlin-Wahlén A: Splanchnic elimination and systemic toxicity of bupivacaine and etidocaine in man. *Acta Anaesthesiol Scand* 1977; 21:521–8
37. Knudsen K, Beckman Suurküla M, Blomberg S, Sjövall J, Edvardsson N: Central nervous and cardiovascular effects of i.v. infusions of ropivacaine, bupivacaine and placebo in volunteers. *Br J Anaesth* 1997; 78:507–14
38. Widman B: Plasma concentration of local anaesthetic agents in regard to absorption, distribution and elimination, with special reference to bupivacaine. *Br J Anaesth* 1975; 47:231–6