

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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Summary Statement: The sink mechanism results in a redistribution of anesthetic from tissues to the blood stream. However, the extent to which this occurs and the associated timescale may not be adequate to explain the dramatic recoveries observed clinically.

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 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 (PBPK) 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 non-toxic dose of bupivacaine. The model was augmented to simulate lipid therapy and extended to the case of accidental intravenous 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 (i) an increase in total plasma concentration, (ii) a decrease in unbound concentration, and (iii) 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 the concentration in the heart falling by 11% within 3 minutes. This initial study suggests that, in isolation, the lipid sink is insufficient to guarantee a reversal of systemic toxicity.

Introduction

Intravenous lipid emulsions (ILEs) show increasing promise as mitigators of systemic toxicity due to lipophilic drug overdose¹⁻⁴. 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 like 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 metabolism of the anesthetic. Also addressed is the partitioning of anesthetic into red blood cells (erythrocytes). The system of differential equations governing the systemic distribution of the anesthetic consists of mass balances based on the following assumptions: (i) clearance of the anesthetic occurs via hepatic metabolism⁵⁻⁸; (ii) tissue disposition is perfusion limited; and (iii) anesthetic in the bloodstream is assumed to be partitioned via rapid equilibrium between 4 sub-compartments: bound to plasma proteins, bound to red blood cells, bound to lipid droplets, or unbound in the aqueous plasma.

Predicted pharmacokinetics are validated by comparison with clinical data from healthy volunteers administered non-toxic 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 intravenous 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 (Figure 1) corresponding to 12 organs and 2 blood compartments (1 arterial, 1 venous). Details of the governing differential equations are provided in Appendix 1.

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 ($\lambda = C_{blood}/C_p$)¹⁷. An alternative approach is employed here. Tucker *et al.* quantified plasma:erythrocyte anesthetic

distribution in blood samples from two healthy individuals⁹. 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 non-linear 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 follows¹⁸.

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

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.

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

Bupivacaine dosage: Dosage of bupivacaine was modeled as a constant rate intravenous 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 Appendix 1). 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: (i) characteristic half-lives; (ii) systemic clearance; and (iii) 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 intravenous injection of 112.5 mg over 3 minutes. 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-second duration of the lipid bolus mimicked that reported by Marwick. After a 3-minute interval, this was followed by a 60 minute 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 1 volume% lipid (20% emulsion diluted with buffer to a composition of 1 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:

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

where $C_{lip,p}$ is the concentration of bupivacaine bound to lipid in plasma. We have chosen here to re-express the binding capacity, B_{max} , as per unit volume of oil ($B_{max} = 0.213$ M; derivation detailed in Appendix 1); LIP is then the time-dependent volume fraction of plasma lipid.

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 (Figure 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]).

^{*} Weinberg, G. L. LipidRescue: Resuscitation for cardiac toxicity. <http://lipidrescue.squarespace.com/>. Last accessed. January 26, 2013.

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

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 intravenous 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 $75.3 \pm 1.32\%$. 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 inter-individual variations in plasma protein binding.

Lipid distribution: *In vitro* experiments have demonstrated that the bulk of bupivacaine uptake by lipid emulsions occurs within one minute of mixing²⁴ – a time similar to that required for lipid to be distributed throughout the bloodstream. Furthermore, ILE droplets have 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-minute intravenous infusion of (i) 29.2 mg, (ii) 44.2 mg, or (iii) 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 (i), plasma concentration data was fitted by the same biexponential model used by Burm *et al.*¹⁰ A weighted non-linear 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 mins and 152 mins for distribution and elimination respectively. Following the approach of Tucker *et al.*⁶, in case (ii), 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^{†11} (Figure 3). 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.

Accidental intravenous 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 (Figure 4A) display maxima at – or shortly following – the end of the bupivacaine infusion for all rapidly perfused organs. A lag of ~10-20 mins is observed for more slowly perfused organs (bone, muscle, skin). The maximum for adipose tissue occurs at ~1.5 hours. The fraction of bupivacaine bound in the blood stream decreases as the anesthetic

[†] data extracted from Tucker *et al.*¹¹ using WebPlotDigitizer (<http://arohatgi.info/WebPlotDigitizer/> ; last accessed January 26, 2013)

concentration increases (Figure 4B). The fraction unbound in plasma increases from ~3.5% to a maximum of ~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 (Figure 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 minutes of ILE therapy, the concentration of bupivacaine in heart tissue is reduced by 11%. Within the first 15 minutes, 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 maximal extent to which tissue concentration is altered (relative to the untreated case) in each of the 12 PBPK organs within the first 15 mins 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.

Blood concentration: The effluent blood from the brain and heart exhibits an increase in bupivacaine concentration upon lipid administration (Figure 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 ($t = 73$ mins) 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 (i) increased quantity of lipid in the blood stream, which would increase the effective lipid binding capacity; or (ii) 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 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 ($K_a = 1504, 3008, 6015, 12030 \text{ M}^{-1}$ 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 minutes of ILE administration. The dependence of this reduction on the lipid binding affinity is logarithmic, such that an increase of 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.*, who reported a PBPK study of liposome-mediated toxicity reversal¹⁷. 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 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 non-linear thereafter⁹. 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 ~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 Berezhkovskiy²⁹. 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 Appendix 1 Table 1).

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 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.* in a study of accelerated efflux from isolated heart models³⁰. 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 (~11%). The concentration of bupivacaine in brain tissue is reduced by a larger extent (18%), but over a longer timeframe (~15 minutes). As the effects of lipid infusions tend to be observed within a few minutes¹, 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 of 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 concentration²⁵, with high bupivacaine concentration (up to ~1000 μM)^{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 buffer²⁴). 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 ~90% of bupivacaine. In whole blood, predicted uptake drops to ~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™) 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 amongst the available binding agents; at high anesthetic concentrations, this principally involves serum albumin and lipid, as the glycoprotein population is saturated at concentrations >30 μM . The redistribution of bupivacaine observed in our model is consistent with modest increases of 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 inter-individual 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 pH¹⁶. 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 serum³⁵) while others have observed pH sensitivity (in buffer²⁴)^{24,26}. Hemodynamic effects, pH effects, hemodilution, and lipid pharmacokinetics will be considered in future implementations of the model.

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Figure Legends

Figure 1. Structure of the PBPK model. 12 organs and two blood compartments are represented in this 14-compartment model.

Figure 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. $K_{AAG,P}$, $K_{HSA,P}$ – protein-plasma partition coefficients. $K_{ILE,P}$ – Lipid-plasma partition coefficient. K_e – erythrocyte-plasma partition coefficient.

Figure 3. Arterial plasma concentration following intravenous delivery of 66.7 mg bupivacaine. The PBPK–predicted curve is plotted alongside the observations of Tucker¹¹.

Figure 4. 112.5 mg intravenous dose of bupivacaine administered over 3 minutes.

(A) Concentration-time curves for the 12 organs included in the 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. PBPK model -- . Data from Tucker⁹ ◇.

Figure 5. 112.5 mg intravenous dose of bupivacaine administered over 3 minutes followed by simulated lipid emulsion therapy (Bolus: 1.5 ml/kg over 1.5 mins; Continuous infusion:

0.25 ml kg⁻¹ min⁻¹ over 60 mins). (A) Normalized concentration-time curves for the 12 organs included in the PBPK model. Abscissa indicates time following the initiation the bupivacaine dose. (B) Bupivacaine concentration curves in heart, brain, and adipose tissues.

Concentrations ($C_{tissue,ILE}$) are expressed relative to those observed for the same anesthetic dose in the absence of lipid ($C_{tissue,untreated}$). Abscissa indicates time following the initiation of lipid

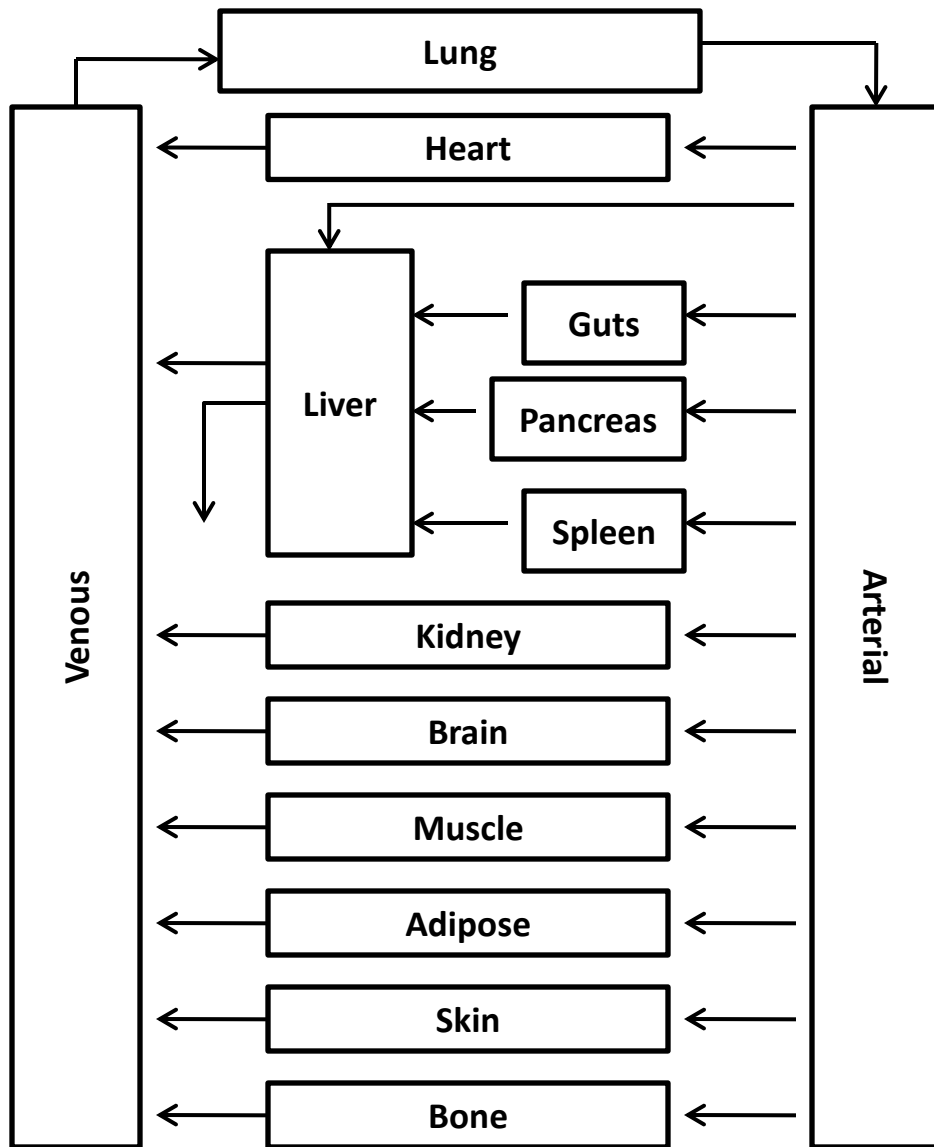
therapy. C – tissue concentration. C_{\max} – maximum concentration in tissue. ILE – intravenous lipid emulsion.

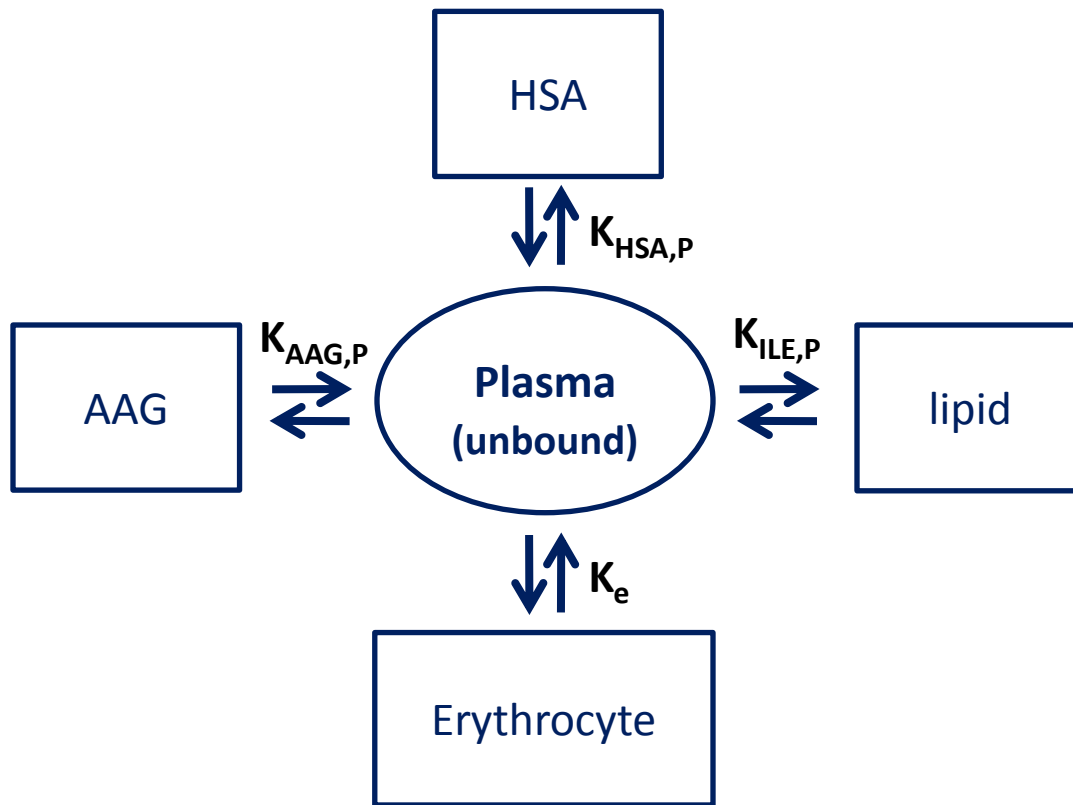
Figure 6. The impact of simulated intravenous lipid emulsion therapy on bupivacaine concentration in tissues during the first 15 minutes of lipid administration. The ordinate indicates to fractional reduction in bupivacaine concentration relative to the case of untreated overdose.

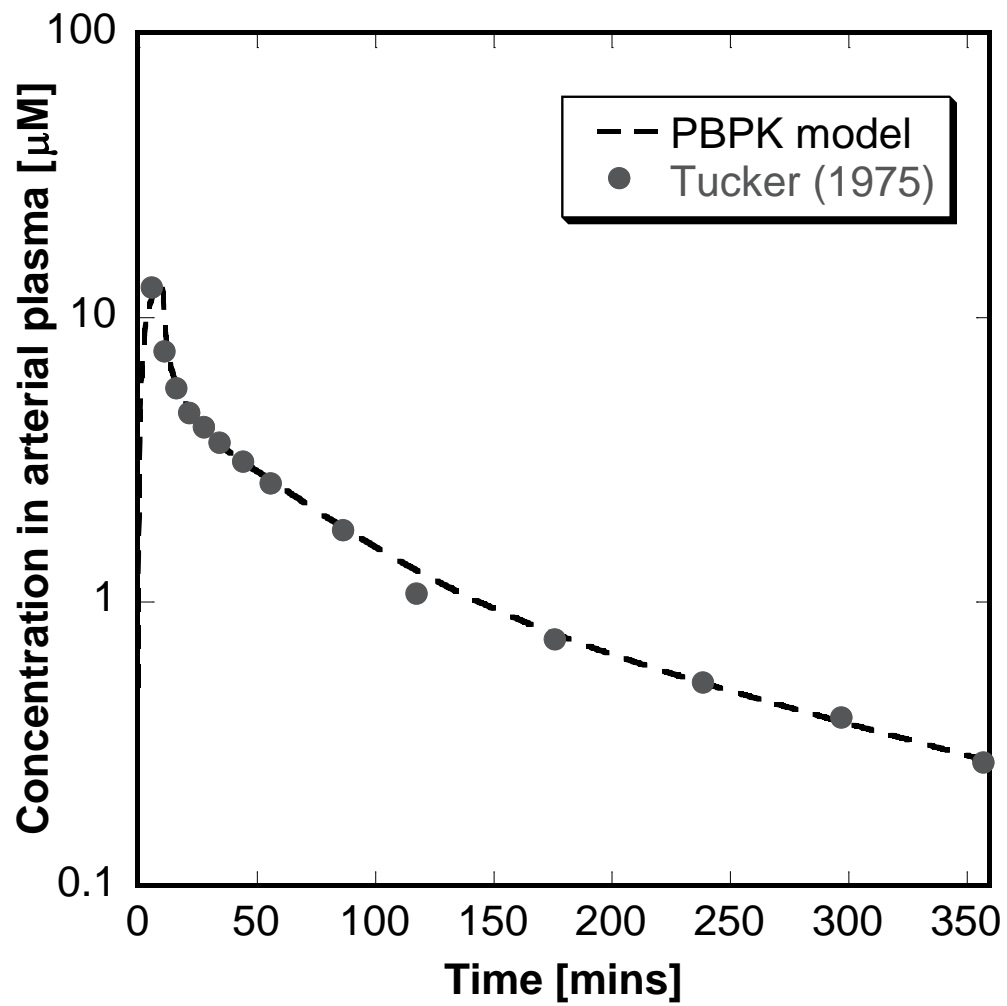
Figure 7. Bupivacaine concentration in effluent blood for the (A) heart and (B) brain. Introduction of lipid (at $t = 8$ mins) causes an increase in blood content of bupivacaine.

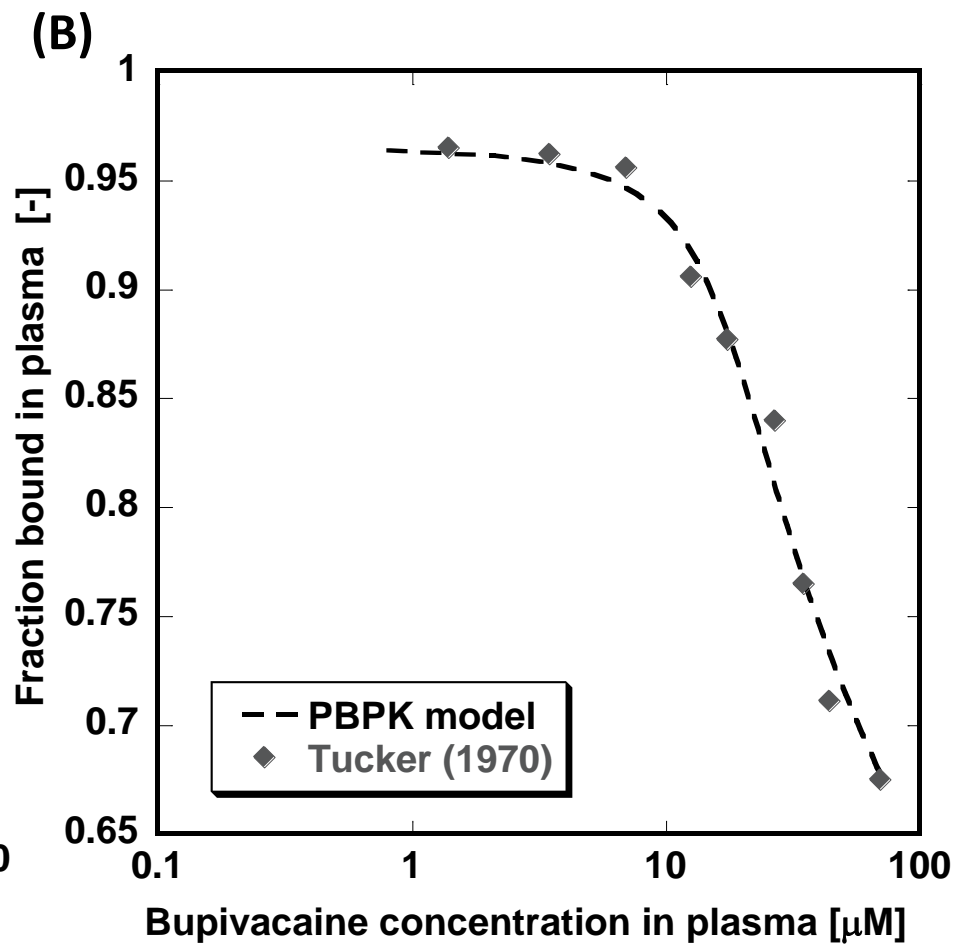
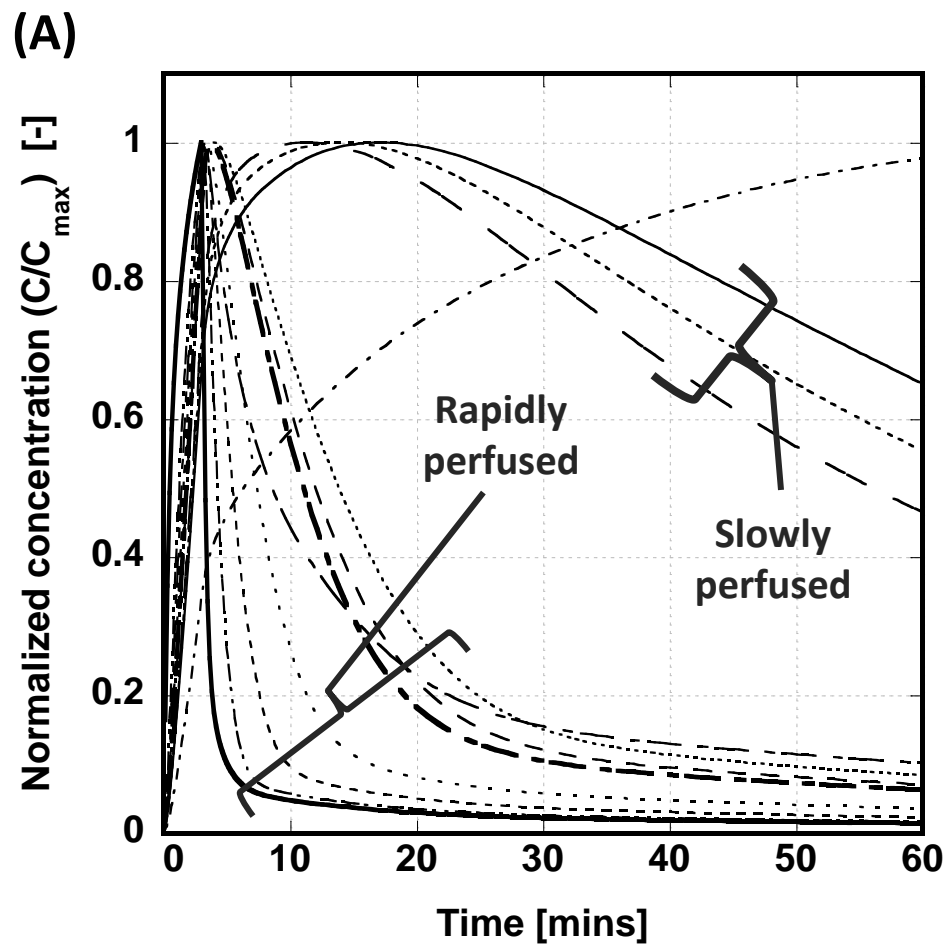
Figure 8. Bupivacaine concentration curves in arterial blood as a consequence of lipid therapy. Concentrations are expressed relative to those observed for the same anesthetic dose in the absence of lipid. Abscissa indicates time following the initiation of lipid therapy. Total concentration in arterial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

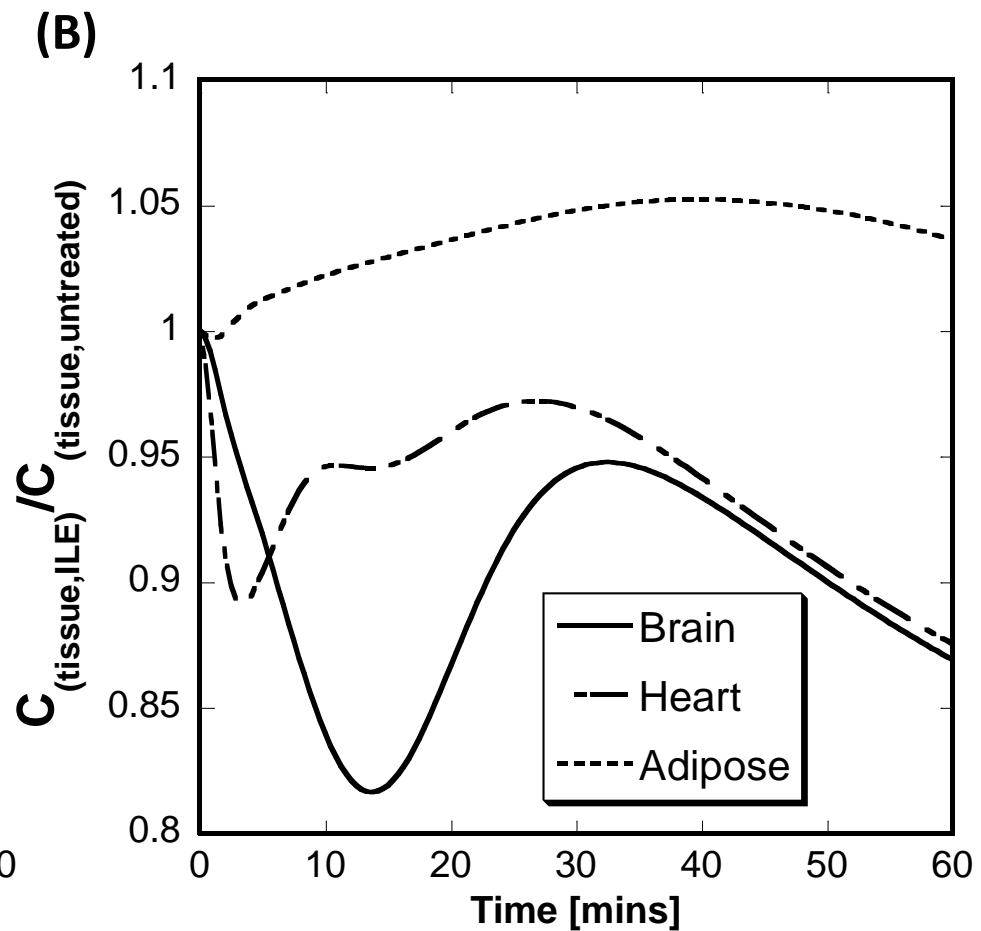
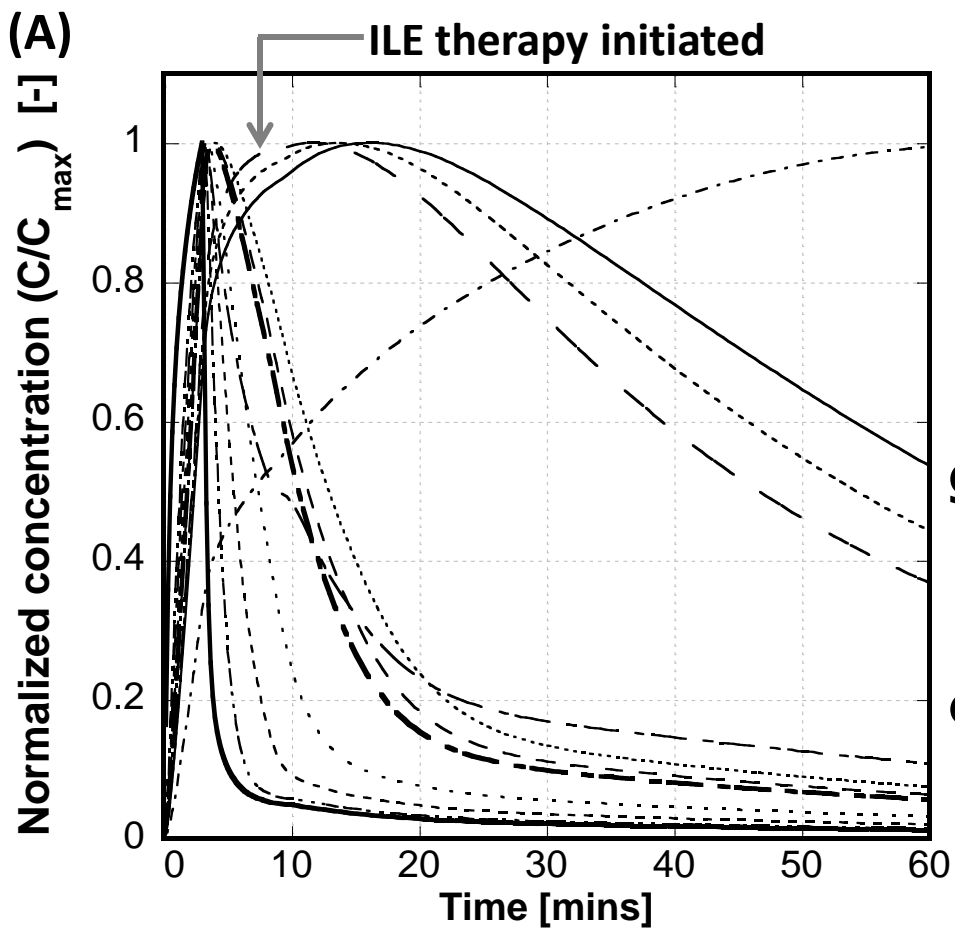
Figure 9. Effect of increased ILE binding affinity. The acute reduction in tissue bupivacaine increases logarithmically as a function of lipid-bupivacaine binding affinity.

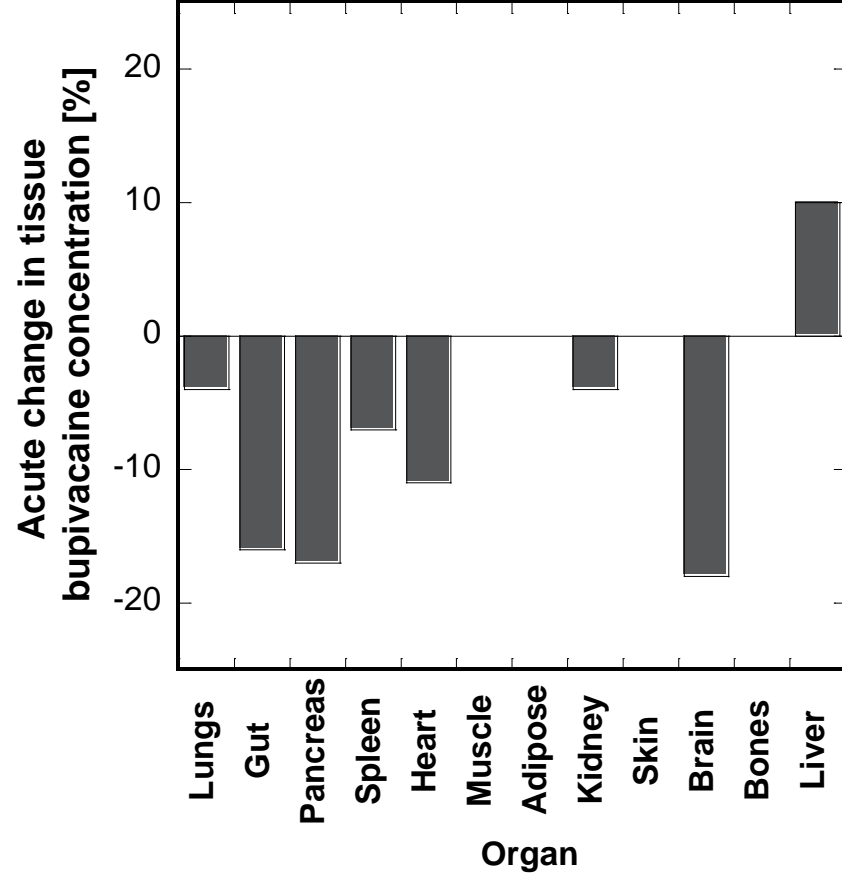


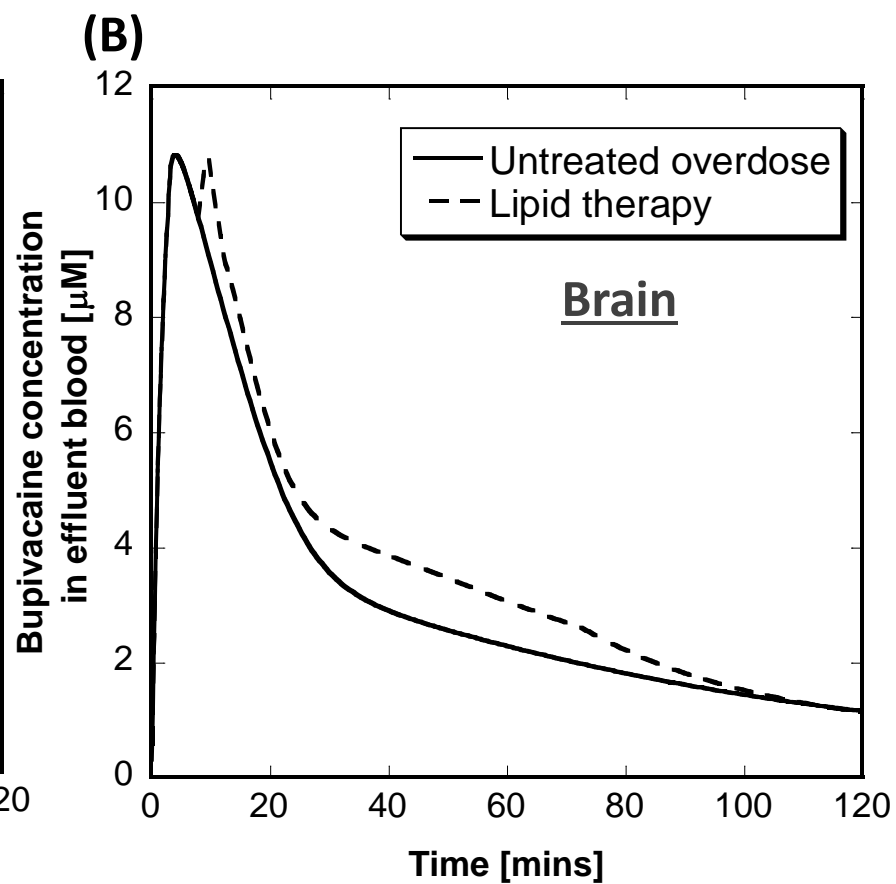
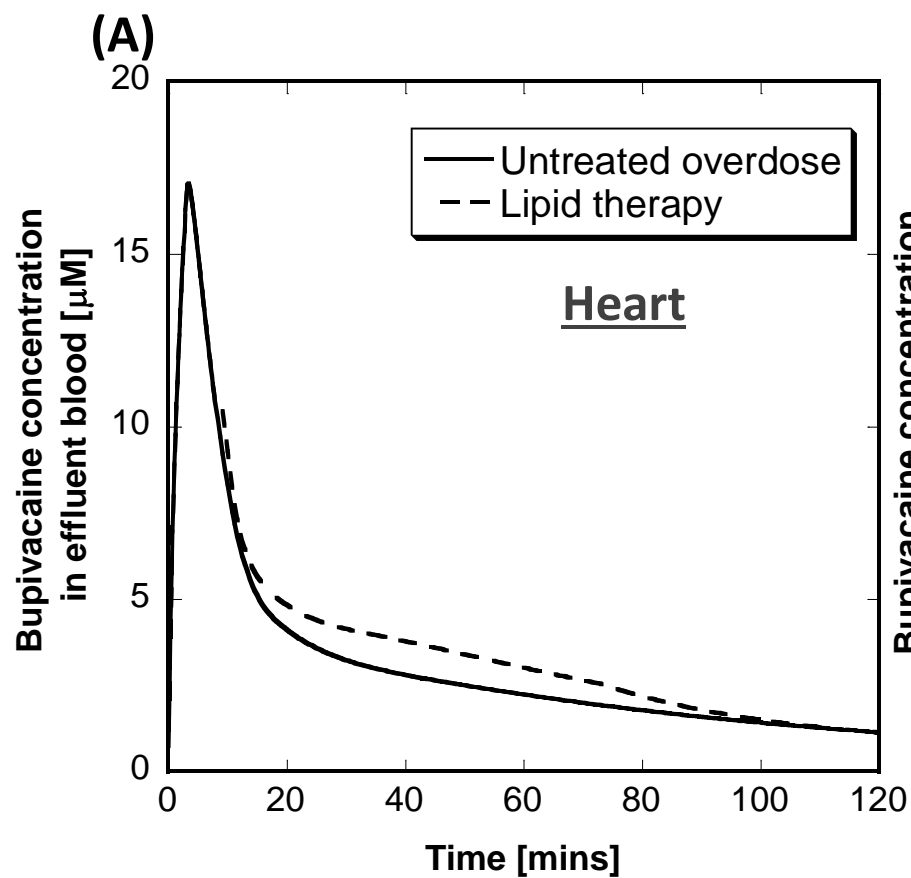


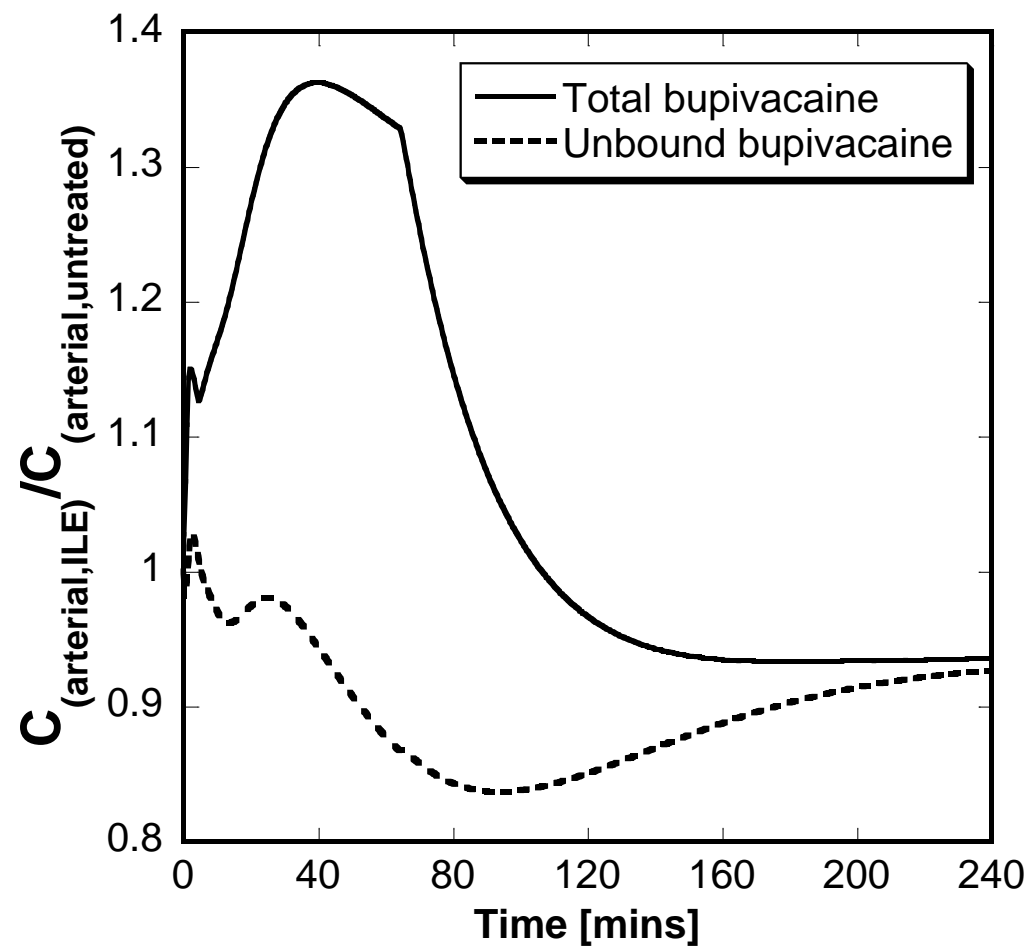


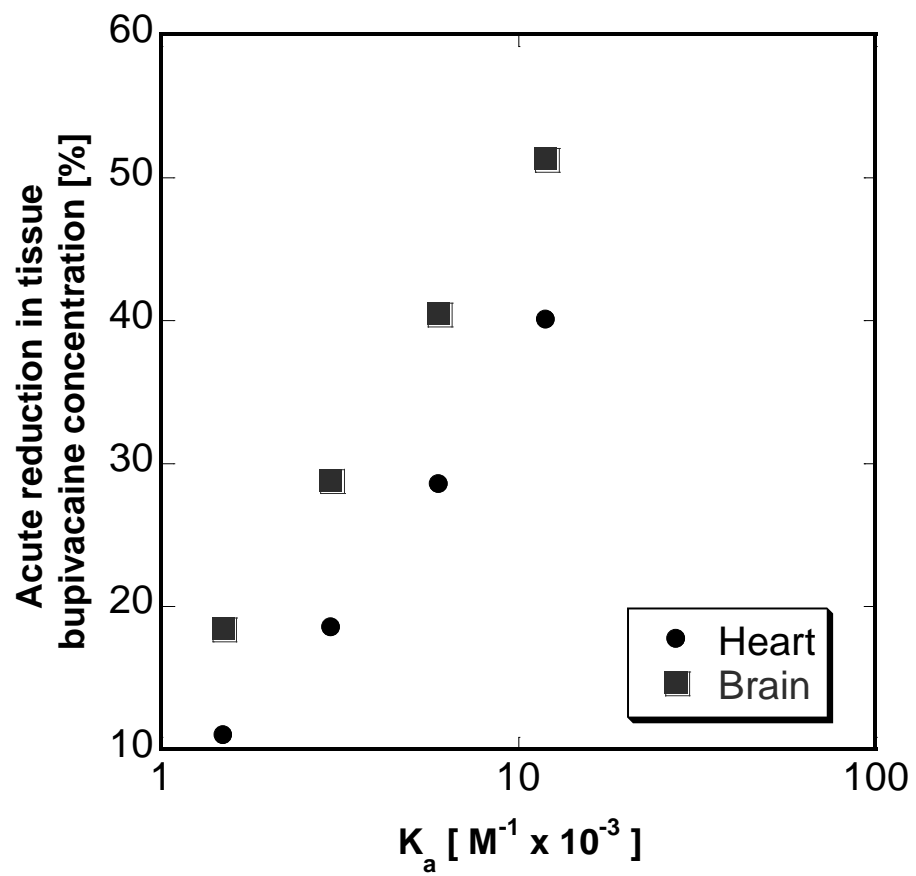












Appendix 1

Mass Balances

Non-eliminating organs (except lungs): For non-eliminating 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],

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

where $i \equiv \text{gut, spleen, pancreas}$, $Cl_{u_{int}}$ is the intrinsic unbound hepatic clearance, and C_{u_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 clearance²².

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

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 $Cl_{u_{int}} = 29.2$ 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.^{5,36,37,38} 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.

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

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

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 sub-compartments. It can be shown that the tissue-plasma and tissue-whole blood partition coefficients are related by:

$$R_{tb} = \frac{R_{tp}}{(1-H)(1+K_{bf})+HK_e} \quad [8]$$

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.

$$\frac{dV_{lip}}{dt} = kV_{lip} + Q \quad [10]$$

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 re-express the binding capacity such that it remains a time independent constant as follows:

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

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).

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

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 Howell et al.¹⁷: In adopting the approach of Howell *et al.*, 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 red blood cell 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:

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

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

The tissue AUC values resulting from the current approach and the prior model are compared in Table 3.