The Effect of Lipid Emulsion on Pharmacokinetics and Tissue Distribution of Bupivacaine in Rats

Kejian Shi, MD,* Yun Xia, MD, PhD,† Quanguang Wang, MD,* Yiquan Wu, MD,* Xiaxi Dong, MD,† Chanjuan Chen, BS,* Wan Tang, BS,* Yujian Zhang, MD,* Mengxu Luo, BS,* Xianqin Wang, PhD,‖ Thomas J. Papadimos, MD, MPH,† and Xuzhong Xu, MD*  

BACKGROUND: While lipid emulsion may reverse the systemic toxicity of bupivacaine, the pharmacokinetics and tissue distribution of bupivacaine after lipid emulsion infusion are not clear. In this study, we assessed the influence of lipid emulsion administration on the pharmacokinetics and tissue distribution of bupivacaine.

METHODS: Rats in the lipid group were administered IV bupivacaine at the rate of 2 mg·kg⁻¹·min⁻¹ for 4 minutes, and then were treated with an infusion of 30% lipid emulsion at the rate of 3 mL·kg⁻¹·min⁻¹ for 5 minutes; saline was substituted in the control group (n = 6 for pharmacokinetics). We then randomly assigned 100 rats into the lipid group and control group (n = 50 for distribution). The toxicity model and treatment were the same as the pharmacokinetic portion. Plasma and tissues including brain, heart, liver, spleen, lung, kidney, omentum, and muscle were collected. The plasma concentration and tissue content of bupivacaine were measured by a liquid chromatography-tandem mass spectrometric method. A 2-compartmental analysis was performed to calculate the pharmacokinetics of bupivacaine.

RESULTS: All data are shown as mean ± SD. After treatment with the lipid emulsion, t1/2 of bupivacaine in the lipid group was significantly shorter (110 ± 25 minutes vs 199 ± 38 minutes, P = 0.001), the clearance was higher (14 ± 4 mL·mg⁻¹·kg⁻¹ vs 9 ± 4 mL·mg⁻¹·kg⁻¹, P = 0.038), and the t1/2α was longer than that of the control group (4 ± 1 minutes vs 2 ± 1 minutes, P = 0.014); the K12 in the lipid group was less than that of the control group (0.13 ± 0.04 vs 0.32 ± 0.13, P = 0.011). In the lipid group, the bupivacaine content in heart, brain, lung, kidney, and spleen was lower than that in the control group, but higher in the liver at 20, 30, and 45 minutes.

CONCLUSION: The lipid sink phenomenon was observed in this study. The use of a lipid emulsion accelerated the elimination of bupivacaine.

an intraperitoneal injection of chloral hydrate (350 mg·kg⁻¹). Tracheal intubation was performed via tracheotomy, and the rats’ lungs were mechanically ventilated with 1% to 2% sevoflurane in 100% oxygen using a rodent volume-controlled ventilator (tidal volume = 8 mL·kg⁻¹, rate = 75–80 breaths·min⁻¹, inspiratory/expiratory ratio = 2.3, HX-300, TME Technology Co, Ltd, Chengdu, China). Body temperature was maintained at 38°C to 39°C with a heating lamp. The left carotid artery was cannulated for arterial blood pressure monitoring and blood sample collection. The left internal jugular vein was cannulated for the infusion of bupivacaine, and the right internal jugular was cannulated for the infusion of the lipid emulsion or saline. Electrocardiography was performed using 3 subcutaneous needle electrodes, and the information was recorded. Hemodynamics and electrocardiographic data were recorded by a MedLab data archiving and retrieval system using U/4C051 (Nanjing Medease Science and Technology Co., Ltd, Nanjing, China). On completion of the invasive procedures, all animals were allowed to stabilize physiologically for 15 minutes.

**Pharmacokinetics**

Twelve male Sprague–Dawley rats were randomly divided into a lipid group and a control group (n = 6). At the end of the stabilization period (baseline time, designated Tb), bupivacaine hydrochloride (Sigma-Aldrich Co., St. Louis, MO) was infused at the rate of 2 mg·kg⁻¹·min⁻¹ for 4 minutes in the left internal jugular vein. Once the infusion of bupivacaine was completed (designated 0 minutes), all rats were infused with either 30% Intralipid (Huarui Pharmaceuticals Co., Ltd., Wuxi, China) or saline at the rate of 3 mL·min⁻¹ for 5 minutes through the right internal jugular vein. Blood samples of 0.5 mL were collected at time 0, 5, 10, 20, 30, 45, 60, 90, 120, 240, and 360 minutes, and the rats were then supplemented with an equal volume of saline (after blood collection). Plasma was separated from blood by centrifugation (4000 rpm, 10 minutes), and stored at −80°C for determination of bupivacaine concentrations.

**Distribution of Bupivacaine**

We randomly assigned 100 male Sprague–Dawley rats into 20 groups (n = 5): lipid5, lipid10, lipid20, lipid30, lipid45, lipid60, lipid90, lipid120, lipid240, lipid360, control5, control10, control20, control30, control45, control60, control90, control120, control240, and control360. Lipid 5 to 360 were the treatment groups that received lipid emulsion infusion; control 5 to 360 were the control groups that received a saline infusion. The toxicity model and treatment were the same as the pharmacokinetic portion of the study. Rats in the lipid 5 to 360 and control 5 to 360 groups were killed at 5, 10, 20, 30, 45, 60, 90, 120, 240, and 360 minutes, respectively. Tissues including brain, heart, liver, spleen, lung, kidney, omentum, and muscle were collected, frozen in liquid nitrogen, and stored at −80°C.

**Measurement of Bupivacaine**

A liquid chromatography-tandem mass spectrometric (Bruker Esquire; Bruker Company, Karlsruhe, Germany) method was developed and validated for the detection of bupivacaine. The parameters of liquid chromatography-tandem mass spectrometry were the same as Chen et al.’s report. Bupivacaine hydrochloride was prepared in a blank plasma or blank tissue homogenate for calibration curves within the range of 0.01 to 40 µg·mL⁻¹ (µg·g⁻¹ for tissue). The samples for recovery and precision measurements were prepared at 0.02, 10, and 40.0 µg·mL⁻¹ (µg·g⁻¹ for tissue) (n = 5, repeated for 5 days).

Frozen tissue samples of 0.2 g were homogenized with 2 mL ultrapure water. Plasma 0.2 mL or 2 mL of tissue homogenate was added to 62.5 ng of ropivacaine as an internal standard, 0.05 M KOH for alkalization, and 1 mL N-hexane and 4 mL acetonitrile for extraction of bupivacaine. The preparations were mixed with a vortex generator for 1 minute and centrifuged at 4000 rpm for 10 minutes. The upper organic phase was neutralized and acidified with 0.3 mL 1.5% HCL solution, and then mixed with a vortex generator for 1 minute, followed by centrifugation at 4000 rpm for 10 minutes. An aqueous phase subnatant of 0.25 mL was taken for the determination of bupivacaine levels.

All of the correlation coefficient values (r²) were >0.995. The lower limit of detection for bupivacaine was 5 ng·mL⁻¹. Recovery of bupivacaine from plasma and the 8 tissues ranged from 91.9% to 112.6%. Intraday and interday relative standard deviations were <6%.

**Pharmacokinetic Calculation**

Pharmacokinetic parameters were calculated by a 2-compartmental analysis model using DAS Ver2.1.1 (Drug and Statistics, Mathematical Pharmacology Professional Committee of China. Shanghai, China). All the data were obtained from the end of bupivacaine infusion until the last sampling time.

Elimination half-life was designated as t1/2 and was calculated using the equation: t1/2 = 0.693/λz. The area under the blood concentration–time curve (AUC) was determined using the trapezoidal method. Apparent volume (V) of distribution was defined as the volume of body fluid that is accounted for when the drug distribution is equivalent to the drug concentration in plasma and was calculated with Vd = Dose /C. CL was defined as the apparent volume of distribution (Vd) removed from the body per unit time and was calculated by the equation: CL = Dose/AUC. K10 represents a rate constant of drug elimination from the central compartment, K12 as that from central compartment to peripheral compartment, and K21 as that from the peripheral compartment to the central compartment.

**Statistical Analysis**

The number of animals in each group was determined based on preliminary study (n = 3). Half-life time of bupivacaine in the lipid and control groups was 106 ± 19 minutes and 194 ± 39 minutes. We assumed a type 1 error protection of 0.05 and a power of 0.90. Six rats were required for each group. All data were analyzed using SPSS 13.0 (SPSS Inc, Chicago, IL) for Windows and presented as mean ± SD. Mean arterial blood pressure and heart rate between the 2 groups were compared by 2-way repeated measures analysis of variance. Pharmacokinetic parameters and bupivacaine content between the 2 groups were compared by independent-sample t test, and corrected P value was chosen when equal
variances were not assumed. \( P < 0.05 \) was considered to be of statistical significance. Curve fitting and bar charts were performed by GraphPad Prism v5.0 (GraphPad Software Inc., San Diego, CA).

**RESULTS**

**Hemodynamics**

Mean arterial blood pressure and heart rate in both groups are shown in Figure 1. There was no statistical significance between the 2 groups (\( P = 0.444 \) for mean blood pressure, \( P = 0.209 \) for heart rate).

**Pharmacokinetics Parameters**

The concentration–time curve between the 2 groups is presented in Figure 2. The comparison of pharmacokinetic parameters between the 2 groups is shown in Table 1. Compared with the control group, the lipid group had an increased \( t_{1/2\alpha} \) \((4 \pm 1 \text{ minutes vs } 2 \pm 1 \text{ minutes}, P = 0.014) \) and bupivacaine elimination \( CL \) \((14 \pm 4 \text{ mL} \cdot \text{mg}^{-1} \cdot \text{kg}^{-1} \text{ vs } 9 \pm 4 \text{ mL} \cdot \text{mg}^{-1} \cdot \text{kg}^{-1}, P = 0.038) \), while exhibiting a reduced \( t_{1/2\beta} \) \((110 \pm 25 \text{ minutes vs } 199 \pm 38 \text{ minutes, } P = 0.001) \) and \( K_{12} \) \((0.13 \pm 0.04 \text{ vs } 0.32 \pm 0.13, P = 0.011) \). The comparison of the apparent \( V_d \), AUC (0–t), AUC (0–\( \infty \)), \( K_{10} \), and \( K_{21} \) between groups demonstrated no statistical significance.

**The Tissue Bupivacaine Content**

Treatment with lipid emulsion decreased the bupivacaine content in the heart, brain, spleen, muscle, and lung. However, the initial bupivacaine content in the liver was increased and was then followed by a reduction in content after 2 hours. The comparison of bupivacaine content between the lipid and control groups, in 8 different organ tissues, is presented in Figure 3.

**DISCUSSION**

Our investigation demonstrated that after infusion of lipid emulsion, bupivacaine elimination half-life and \( K_{12} \) decreased, the \( CL \) rate increased, distribution half-life was prolonged, and the content in brain, myocardium, lung, kidney, spleen, and muscle was reduced. However, the bupivacaine content in the liver was increased at the time points of 20, 30, and 45 minutes.

In this study, we chose a model of bupivacaine toxicity without cardiac arrest. We resolved that using a cardiac arrest model would lead to a scenario of prolonged duration of recovery and a variability in hemodynamics, especially in the face of the infusion of lipid emulsion for resuscitation whereby the determination of bupivacaine metabolism and distribution, i.e., the pharmacokinetics,

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**Table 1. Comparison of Pharmacokinetics Between the Lipid and Control Groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lipid group</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{1/2\alpha} ) (min)</td>
<td>4 ± 1</td>
<td>2 ± 1</td>
<td>0.014</td>
</tr>
<tr>
<td>( t_{1/2\beta} ) (min)</td>
<td>110 ± 25</td>
<td>199 ± 38</td>
<td>0.001</td>
</tr>
<tr>
<td>( V_d ) (L·kg(^{-1}))</td>
<td>0.26 ± 0.05</td>
<td>0.24 ± 0.05</td>
<td>0.456</td>
</tr>
<tr>
<td>( CL ) (mL·min(^{-1})·kg(^{-1}))</td>
<td>14 ± 4</td>
<td>9 ± 4</td>
<td>0.038</td>
</tr>
<tr>
<td>AUC (0–t) (mg·L(^{-1})·min(^{-1}))</td>
<td>588 ± 170</td>
<td>800 ± 371</td>
<td>0.233</td>
</tr>
<tr>
<td>AUC (0–( \infty )) (mg·L(^{-1})·min(^{-1}))</td>
<td>640 ± 199</td>
<td>1123 ± 620</td>
<td>0.099</td>
</tr>
<tr>
<td>( K_{10} ) (1/min)</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.111</td>
</tr>
<tr>
<td>( K_{12} ) (1/min)</td>
<td>0.13 ± 0.04</td>
<td>0.32 ± 0.13</td>
<td>0.011</td>
</tr>
<tr>
<td>( K_{21} ) (1/min)</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.03</td>
<td>0.170</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD. For each group, \( n = 6 \).

\( t_{1/2} = \) elimination half-life; \( V_d = \) volume of distribution; \( CL = \) clearance; \( AUC = \) area under the blood concentration–time curve; \( K_{10} = \) rate constant of drug elimination from the central compartment; \( K_{12} = \) rate constant of drug elimination from central compartment to peripheral compartment; \( K_{21} = \) rate constant of drug elimination from the peripheral compartment to the central compartment.

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**Figure 1.** Mean and 95% confidence intervals of heart rate and mean arterial blood pressure of the lipid and control groups are shown during Tb to 360 minutes, \( n = 6 \). There is no statistically significant difference of heart and blood pressure between the groups.

**Figure 2.** A. Individual plasma concentration–time profile in 6 rats in the lipid group. B. Individual plasma concentration–time profile in 6 rats in the control group. All curves are on semilogarithmic coordinates. The value in Y axis is the logarithm of value of plasma concentration.
would become exceedingly complicated. Although cardiovascular complications such as arrhythmia or hypotension may manifest when bupivacaine is infused at the rate of 2 mg·kg⁻¹·min⁻¹ for 4 minutes (in our study model), recovery can occur promptly when such an infusion is discontinued, or when an infusion of lipid (or saline) is initiated.

In the early stage of the end of lipid emulsion infusion, the bupivacaine concentration of plasma increased and the t₁/₂α became prolonged, this implies that there were increased levels of bupivacaine in plasma. Additionally, the value of K₁₂ demonstrated that lipid emulsion limited the migration of bupivacaine from the central compartment to the peripheral compartment, which confirmed the effectivity of the lipid sink theory. It would seem intuitive that the Vd should be reduced, but, in fact, the Vd of the lipid group was not reduced compared with the control group. This may be attributed to the complex change of plasma concentration. Although the plasma concentration was increased by the lipid sink effect in the early stages, the liver content and elimination of bupivacaine were increased in regard to the hepatic pharmacologic trends of lipid emulsion. This led to a rapid decrease in plasma concentration, which offset the effect of the early increase in plasma concentration on Vd. Long-chain triglycerides, the main components of lipid emulsion, have an affinity for the liver. Bupivacaine is a liposoluble drug, binding tightly to a lipid emulsion. After lipid emulsion infusion, the contents of bupivacaine in the liver at 20, 30, 45 minutes were higher than the control group, suggesting that the liver may be facile in its uptake of bupivacaine because of its long-chain triglyceride (cytochrome P450 is the primary catabolic enzyme of bupivacaine). This increase of bupivacaine in the liver may have caused an acceleration of metabolism and may be the explanation as to why the elimination half-life of the lipid group was shorter, as well as the reason for its increased CL.

Litonius et al. showed that IV lipid emulsion reduced the half-life of bupivacaine, and Litz et al. reported that infusion of lipid emulsion accelerated the decline of mepivacaine and prilocaine levels. Our results are consistent with both of these studies. A comparison of pharmacokinetic parameters between the 2 groups in our study indicated that lipid emulsion played a significant role in the reduction of bupivacaine toxicity and the prevention of the recurrence of toxicity after resuscitation.

The content of bupivacaine in tissues of the myocardium and brain affects toxicity. Thus, the key to recovery is to reduce the content of bupivacaine. In this study, there were significant decreases of bupivacaine content in the heart and brain after administration of lipid emulsion. The average content in the myocardium was about half of that in the control group and subsequently was maintained at a low level, which is consistent with previous reports. Therefore, we can also infer that lipid emulsions reduce central nervous system toxicity because of the decreased content in brain tissue.

Furthermore, the content of bupivacaine in other tissues seems to be related to organ perfusion. The high content found in the lung and kidney is attributed to their abundant blood perfusion. The pulmonary system is the first organ mass that lipid emulsion passes through, so the earliest decrease in bupivacaine content may be demonstrated here. This early decrease in the content of bupivacaine as it passed through the lungs was more evident in the treatment group as compared with the control group, and the concomitant weakening of the influence of the lungs at the end of the infusion was also evident. At 60 minutes, the content of bupivacaine in the lung was lower than that in control
group, again due to accelerated elimination. The variation of bupivacaine in the kidney is similar to that of the lung. Although a decline of bupivacaine in lung and kidney was observed, the lipid sink effect played an important role in the early stages, but accelerated elimination was predominant in the later stages. There was no lipid sink phenomenon observed in the spleen, muscles, and the omentum in the early stage of bupivacaine toxicity. Nevertheless, the accelerated elimination decreased the concentration of bupivacaine in the latter stage, except for the omentum.

A weakness of our study design is that we measured the total concentration of bupivacaine in plasma, but not its free concentration. The model used in this study is quite different from the asystole models used previously, of which the pharmacokinetics and distribution are more complicated because of the diversity of survival rate and hemodynamics after cardiopulmonary resuscitation. This is a study design difference that must be taken into consideration when interpreting our data.

In this study, we have demonstrated that after an infusion of lipid emulsion, the content of bupivacaine in the liver increases, as well as the CL of bupivacaine, and its half-life decreases. The lipid sink effect and accelerated elimination play an important role in the distribution of bupivacaine in rats, both of which are important mechanisms of action of lipid emulsions in the reversal of the systemic toxicity of bupivacaine.

DISCLOSURES
Name: Kejian Shi, MD.
Contribution: This author helped design and conduct the study, analyze the data, and write the manuscript.
Attestation: Kejian Shi has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Yun Xia, MD, PhD.
Contribution: This author helped design the study and write the manuscript.
Attestation: Yun Xia has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Quanguang Wang, MD.
Contribution: This author helped design the study, analyze the data, and write the manuscript.
Attestation: Quanguang Wang has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Yiquan Wu, MD.
Contribution: This author helped conduct the study and write the manuscript.
Attestation: Yiquan Wu has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Xiaoxi Dong, MD.
Contribution: This author helped conduct the study and study data collection.
Attestation: Xiaoxi Dong has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Chanjuan Chen, BS.
Contribution: This author helped conduct the study.
Attestation: Chanjuan Chen has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

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9. Litz RJ, Roessel T, Heller AR, Stehr SN. Reversal of central nervous system and cardiac toxicity after local

Lipid in Pharmacokinetics and Distribution of Bupivacaine

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Name: Yun Xia, MD, PhD.
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Name: Quanguang Wang, MD.
Contribution: This author helped design the study, analyze the data, and write the manuscript.
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Name: Yiquan Wu, MD.
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Attestation: Yiquan Wu has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Contribution: This author helped conduct the study and study data collection.
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