High-Dose Bupivacaine Remotely Loaded into Multivesicular Liposomes Demonstrates Slow Drug Release Without Systemic Toxic Plasma Concentrations After Subcutaneous Administration in Humans

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BACKGROUND: Depot formulations prolong the analgesic effect of local anesthetics and reduce peak plasma drug concentration. This allows for safer administration of larger doses of local anesthetics, which further prolongs the duration of analgesic effect. We previously reported the development of large multivesicular vesicles (LMVVs) remotely loaded with bupivacaine (LMVV liposomal bupivacaine) and demonstrated a >5-fold prolongation of analgesic effect in animals and humans. In this study, we present pharmacokinetic data of LMVV liposomal bupivacaine in humans.

METHODS: Healthy volunteers received subcutaneous injections of 20 mL plain 0.5% bupivacaine and, 1 week later, 20 mL of 2% LMVV liposomal bupivacaine in a prospective, open-label, crossover, controlled study.

RESULTS: Eight subjects were studied. No subjective side effects of local anesthetics were observed. The maximal plasma concentration and the time to achieve maximal plasma concentration were assessed by modeling plasma concentration–time profiles. Maximal plasma concentration was not significantly different between groups (0.87 ± 0.45 μg/mL and 0.83 ± 0.34 μg/mL for plain and liposomal bupivacaine, respectively; P = not significant, 0.83). These values are well below the putative toxic plasma concentration of 2 to 4 μg/mL. Time to achieve maximal plasma concentration was 7-fold greater for the liposomal preparation (262 ± 149 minutes vs 37.5 ± 16 minutes, P < 0.01).

CONCLUSIONS: Peak plasma bupivacaine concentrations were not different in the 2 groups, despite a 4-fold increase in total bupivacaine dose administered in the novel liposomal preparation. The delayed elimination and prolonged redistribution of liposomal bupivacaine to plasma is compatible with the depot-related slow-release effect leading to the prolonged pharmacodynamic effect previously reported. (Anesth Analg 2010;110:1018–23)
bupivacaine that had been reported in animals^2–4,12 and in humans.\(^{23}\)

For the first hour after drug administration, subjects were monitored (continuous 3-lead electrocardiogram and noninvasive arterial blood pressure) in a postanesthesia care unit.

**Preparation of Liposomes**

The LMVV liposomal bupivacaine used in this study was identical to that described previously,\(^{10}\) and the preparation and in vitro characteristics have been described in detail.\(^{10}\) The bupivacaine-loaded LMVs had a D/PL ratio of 1.8, which was achieved using the remote (active) loading of high concentrations of bupivacaine by means of an intraliposome-high/extraliposome-medium-low transmembrane ammonium sulfate gradient into LMVs. LMVV liposomal bupivacaine is characterized by a large intraliposomal multicompartment aqueous phase with a high capacity for bupivacaine loading. As described in previous reports,\(^{10,11}\) the product was evaluated for size distribution (2439 ± 544 nm) by photon correlation spectroscopy, bupivacaine concentration in all preparations including control was confirmed by high-performance liquid chromatography, sterility was confirmed by lack of growth after 2 weeks in aerobic and anaerobic culture media, and the lack of pyrogenticity was confirmed using the limulus test.\(^{10,11}\) Drug-release characteristics have been reported previously.\(^{10}\) LMVV liposomal bupivacaine was diluted in normal saline to yield formulations of 0.5\%, 1.0\%, 1.5\%, and 2\% bupivacaine, which were stored at 4°C and dispensed by a pharmacist. Standard 0.5\% bupivacaine (5 mg/mL; Abbott Laboratories, North Chicago, IL) was used as control.

**Determination of Plasma Bupivacaine Concentration**

After plain bupivacaine administration, blood was sampled to determine the plasma bupivacaine concentration at baseline and at 15, 30, 45, 60, 90, 120, 180, 240, 480, 1440, 1920, and 2880 minutes in all subjects. Venous blood was sampled via a 16-gauge IV catheter in the forearm after the aspiration of 10 mL of dead space. Sampled blood was collected into heparinized vials. Plasma was immediately separated from blood cells by low-speed centrifugation, and samples of 2 mL plasma were labeled and stored frozen at −20°C until chromatographic analysis. Bupivacaine was extracted from plasma samples and analyzed using high-performance liquid chromatography, as described previously.\(^{11}\) Mepivacaine was used as an internal standard. The limit of quantification of bupivacaine plasma concentration was 5 ng/mL.

**Pharmacokinetic Assessment**

The concentration-time data obtained in the study were analyzed by the noncompartmental approach. The following pharmacokinetic parameters were calculated: peak plasma bupivacaine concentration (Cmax), the time to achieve the maximal plasma concentration (Tmax), the area under bupivacaine time-concentration curve extrapolated...
to infinity (AUC), and the terminal half-life. In addition, the concentration-time data of bupivacaine after IV administration was obtained from a previously published study (as described in detail in the Appendix), which allowed the calculation of absolute bioavailability values of bupivacaine after subcutaneous administration. The calculations were performed using WinNonlin software (WinNonlin version 4.5, Pharsight Corporation, Mountain View, CA).

**Statistical Analysis**

Normally distributed data are presented as mean ± SD; nonnormally distributed data are presented as median (95% confidence interval). In view of the small size of the data set, an appropriate nonparametric test (Wilcoxon signed rank test) was used to detect significant differences between the pharmacokinetic parameters for 0.5% plain bupivacaine and 2% liposomal bupivacaine. Significance was assumed at $P \leq 0.05$.

**RESULTS**

Eight subjects were studied. The mean age was 33 years (range, 26–44 years), mean height was 176 cm (range, 165–185 cm), and mean weight was 78 kg (range, 60–110 kg). No subject reported symptoms of local anesthetic toxicity. Plasma bupivacaine concentration did not exceed 2 g/mL in any of the subjects. All data sets were normally distributed and were expressed as mean and SD.

**Pharmacokinetic Parameters**

The plasma bupivacaine concentration at time intervals after subcutaneous administration of 20 mL of 0.5% plain bupivacaine and 20 mL of 2% LMVV liposomal bupivacaine is shown in Figure 1, a–c. The pharmacokinetic parameters for the plain and LMVV liposomal bupivacaine after subcutaneous administration are listed in Table 1. The Cmax was not significantly different between groups. The highest measured plasma bupivacaine concentration for any subject was 1.51 g/mL, recorded 30 minutes after the administration of 0.5% plain bupivacaine (Fig. 1b). The highest measured plasma bupivacaine concentration for an individual subject in the liposomal bupivacaine group was 1.36 g/mL, recorded at 90 minutes (Fig. 1c). Modeled Cmax data for different individuals ranged from 0.4 to 1.5 µg/mL and 0.3 to 1.3 µg/mL for plain and liposomal bupivacaine, respectively. These values are well below the putative toxic plasma concentration reported in humans of 2 to 4 µg/mL [28].

Comparing the plain and liposomal bupivacaine preparations, there was no difference in the modeled Cmax, despite a 4-fold increase in total bupivacaine dose in the liposomal preparation whereas the modeled Tmax was 7-fold greater in the liposomal preparation (262 ± 149 minutes vs 37.5 ± 16 minutes in the plain bupivacaine preparation, $P < 0.01$). The extrapolated area at the tail of the plasma bupivacaine concentration–time curve was 28.2% ± 12.6% for plain bupivacaine and 26.3% ± 22.3% for LMVV liposomal bupivacaine. The calculated absolute bioavailability from subcutaneous injection was 87% and 204% for plain and liposomal bupivacaine, respectively. The terminal half-life of plain bupivacaine after IV administration obtained from the literature (67.3 minutes) was shorter than the half-life after subcutaneous administration of plain (131 ± 58 minutes) or liposomal bupivacaine (1294 ± 860 minutes).

**DISCUSSION**

We report pharmacokinetic data in humans using clinically relevant doses of liposomal bupivacaine remotely loaded...
The pharmacokinetic study showed no difference in Cmax between standard and liposomal bupivacaine, despite a 4-fold increase in bupivacaine dose for the liposomal preparation.

The threshold plasma concentrations of bupivacaine that are reported to be associated with the onset of central nervous system toxicity are in the range of 2 to 4 μg/mL. We demonstrated a Cmax range of 0.4 to 1.4 μg/mL for 20 mL of 2% LMVV liposomal bupivacaine, a plasma concentration range that is well below the reported putative toxic plasma concentration.

Rapid local redistribution and systemic absorption of plain local anesthetics curtails the local anesthetic effect and increases plasma drug concentration, which may lead to systemic toxicity. Slow-release local anesthetics prolong drug action at the effect site, both prolonging anesthetic effect and reducing systemic absorption. The liposomal bupivacaine preparation had properties typical of slow-release formulations, resulting in 7-fold prolongation of Tmax. Although we were unable to measure injection-site concentrations of bupivacaine delivered via the liposomal preparation in this study, the delayed redistribution of liposomal bupivacaine to plasma serves as a good indication for the depot-related slow-release profile of bupivacaine delivered locally via liposomes and is compatible with the prolonged pharmacodynamic effect previously reported in animals and humans.

Availability of the concentration-time data for bupivacaine after IV administration from previously published research allowed us to calculate the extent of absorption of subcutaneously administered bupivacaine. The plain bupivacaine formulation demonstrated a high absolute bioavailability of approximately 87%. Interestingly, the apparent absolute bioavailability of the liposomal formulation was >200%. The simple comparison of the AUC and the apparent difference in bioavailability for plain and liposomal bupivacaine suggests that the elimination pharmacokinetics of liposomal bupivacaine is not linear with respect to dose in a simple aqueous media. This finding is most likely to be attributed to a direct absorption of a fraction of liposomes (presumably the smallest liposomes <400 nm) into the systemic circulation (probably via lymphatic and/or venous drainage). The presence of even a small amount of liposomes in the circulation would reduce the systemic clearance of bupivacaine and result in the increased AUC that was observed in the study. The processes that we hypothesize to occur are summarized in Figure 2.

To more thoroughly investigate the absorption kinetics of plain bupivacaine after subcutaneous administration, deconvolution was performed using the pharmacokinetic parameters of IV bupivacaine in a previously published study obtained by compartmental analysis (as described in the Appendix). As can be seen from Figure 3 and in the Appendix, >50% of the dose was absorbed into the systemic circulation during the first hour after subcutaneous administration of plain bupivacaine. Because systemic clearance of the drug after administration of the liposomal

### Table 1. Pharmacokinetic Parameters of Plain and Liposomal Bupivacaine in Humans, Assessed by Noncompartmental Analysis

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>20 mL, 0% plain bupivacaine</th>
<th>20 mL, 2% liposomal bupivacaine</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (μg/mL)</td>
<td>0.87 ± 0.45</td>
<td>0.83 ± 0.34</td>
<td>NS (P = 0.83)</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>37.5 ± 16</td>
<td>262 ± 149</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>AUC (min · μg/mL)</td>
<td>150 ± 74.1</td>
<td>1410 ± 759</td>
<td>NA</td>
</tr>
<tr>
<td>Terminal half-life (min)</td>
<td>131 ± 58</td>
<td>1294 ± 860</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

For details see text. Data are ± SD. NA (not applicable): comparing AUCs for statistical significance is not relevant in this case, because of different doses. Cmax = peak plasma bupivacaine concentration; Tmax = time to achieve the maximum plasma concentration; AUC = area under the curve.
formulation is apparently altered, the IV data of plain bupivacaine cannot be used to calculate either the extent of absorption or the absorption function for the liposomal formulation. The pharmacokinetic parameters required for deconvolution could be obtained by performing a separate study of IV administered liposomal bupivacaine formulation; however, this would not contribute directly to the aim of our current study, which is to show the lack of systemic toxic levels after subcutaneous administration of high-dose liposomal bupivacaine.

In a previous study, we demonstrated a prolonged analgesic effect after the administration of 0.5 mL of 2% liposomal bupivacaine for 48 hours. In that study, we reported a double-blind, randomized crossover trial in which the efficacy of LMVV liposomal bupivacaine was studied in volunteers. The volunteers received simultaneous intradermal injections at 4 different skin locations with 0.5 mL each of standard (nonliposomal) 0.5% bupivacaine and of 0.5%, 1%, and 2% bupivacaine in the form of LMVV liposomal bupivacaine. The 0.5%, 1%, and 2% LMVV liposomal bupivacaine provided 19, 38, and 48 hours of analgesia to pinprick, respectively, compared with 1 hour for the standard 0.5% bupivacaine control. However, the simultaneous, multiple-site, crossover design of that study and the miniscule volumes of local anesthetic used made pharmacokinetic assessment impossible.

Although we report the AUC for the plasma bupivacaine concentration–time curve, this is a less useful measure of drug efficacy or side effects in this scenario, because drug efficacy is dependent on bupivacaine concentration at the site of injection (and not on plasma bupivacaine concentration), whereas side effects are dependent on the maximal plasma bupivacaine concentration (Cmax) (and not on the AUC of the plasma bupivacaine concentration–time curve). Furthermore, the AUC data in this study were extrapolated beyond 15%, and therefore may be inaccurate.

Measurement of plasma bupivacaine concentrations at intervals in the elimination tail would have enabled an assessment of the bioavailability of plain and liposomal bupivacaine, administered subcutaneously; however, this was not the primary objective of this study.

The order of drug administration was not randomized in this study because the previously reported prolonged elimination time of liposomal bupivacaine in both animals and in humans suggested that we would have had to wait for an inordinately long period of time for complete washout of liposomal bupivacaine before administration of the aqueous drug. Indeed, the plasma bupivacaine concentrations at baseline were negligible on both days. Although the order of drug administration was not performed in a blinded manner, the technician performing the assays was blinded. Consequently, this does not present a serious limitation to the conclusions of this study.

The sustained release of bupivacaine from microparticles has been associated with myotoxicity after IM injection. Before advocating the use of LMVV liposomal bupivacaine for peripheral or neuraxial blockade, the lack of neurotoxicity from either the prolonged local anesthetic action or from the liposomal preparation must be demonstrated.

This study demonstrates that the liposomal formulation allows the administration of a 4-fold increase in bupivacaine, without increasing peak plasma concentrations or the risk of systemic toxicity, and that the redistribution of drug to plasma is extended over a time range compatible with the prolonged analgesic effect previously described.

DISCLOSURE

YB holds patent rights for this formulation. In the event of future commercial application of liposomes, New York University School of Medicine, Yissum R&D Company of the Hebrew University, and YB could potentially receive royalties.

APPENDIX

In our study, pharmacokinetic data after IV administration of bupivacaine were not evaluated because we compared 2 formulations both administered via the subcutaneous (SC) route. To obtain the absorption function for SC bupivacaine, we needed to know the distribution and elimination of the compound after IV administration as a reference control. We performed a literature search to find a suitable data series to serve as an IV control. Information on systemic pharmacokinetics of IV bupivacaine is limited; however, we found a reference for a study that compared pharmacokinetics of levobupivacaine and bupivacaine in volunteers after IV administration. In that study, 12 healthy male volunteers received 40 mg bupivacaine as an 8-minute IV infusion. The mean concentration-time data were captured by computer digitalization. The data were adequately described by the classical 2-compartment pharmacokinetic model using a uniform weighting scheme (where the plasma concentration [C] is described by the following equation: C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}). Figure 3 shows the original data set together with the prediction provided by the model. The pharmacokinetic parameters were as follows: \( \alpha = 0.115 \text{ min}^{-1} \), \( \beta = 0.0106 \text{ min}^{-1} \), \( A = 1.50 \mu g/mL \), \( B = 0.585 \mu g/mL \), and the volumes of the central and the peripheral compartments were 19.1 and 26.5 L, respectively.
These parameters were used to describe the unit impulse response function to perform the numerical deconvolution on the concentration-time profile of plain bupivacaine from this study. Figure 4 presents the percent of the absorbed dose as a function of time for plain bupivacaine. Deconvolution could not be used to estimate the absorption function of liposomal bupivacaine because linearity with respect to dose is apparently violated in this case.

REFERENCES

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