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Kinetic and dynamic studies of liposomal bupivacaine and bupivacaine solution after subcutaneous injection in rats

Hsiu-Ying Yu, Shyh-Dar Li and Pin Sun

Abstract

The pharmacodynamics and pharmacokinetics of bupivacaine in solution and in liposome preparations following subcutaneous administration were studied in rats. Multilamellar vesicles entrapping bupivacaine solution were prepared. The local anaesthetic effect was estimated by the tail-flick test in Wistar rats treated with 1 mg bupivacaine in 0.2-mL preparations. Plasma concentrations of bupivacaine were determined by high-performance liquid chromatography. The results showed that both bupivacaine solution and bupivacaine liposomes revealed local anaesthetic effects in the initial tail-flick test (15 min after injection). With bupivacaine liposomes, the duration of action was 5-fold (447±28.9 vs 87±6.7 min), the maximum possible effect was 2-fold (100±0 vs 47.6±13%), and the peak plasma concentration (C_{max}) was less than one-fifth (0.12±0.04 vs 0.65±0.04 μg mL^{-1}) that with bupivacaine solution. The sensory block effect of bupivacaine solution completely resolved at 90 min, while the plasma concentration of bupivacaine was still more than half the C_{max}. Bupivacaine liposomes resulted in a low and relatively constant plasma level (approx. 0.1 μg mL^{-1}) and a pronounced local anaesthetic effect throughout the experimental period (> 7 h). In conclusion, bupivacaine liposomes elevated the intensity and prolonged the duration of the local anaesthetic effect of bupivacaine, and suppressed the systemic absorption rate of encapsulated bupivacaine.

School of Pharmacy College of Medicine, National Taiwan University, 1, Jen-Ai Road, Section 1, Taipei 100, Taiwan, Republic of China

Hsiu-Ying Yu, Shyh-Dar Li, Pin Sun

Correspondence: Hsiu-Ying Yu, School of Pharmacy College of Medicine, National Taiwan University, 1, Jen-Ai Road, Section 1, Taipei 100, Taiwan, Republic of China. E-mail: ying@ha.mc.ntu.edu.tw

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Introduction

Bupivacaine, an amide-type local anaesthetic, is widely used for infiltration and regional anaesthesia. However, its undesirable effects of central nervous system and cardiac toxicity can cause severe problems (Bloci & Covino 1981; Catterall & Mackie 1996). It appears to be more cardiotoxic than other local anaesthetic agents. Since these toxic effects are directly related to the concentration of the drug in systemic circulation, inhibition of systemic drug absorption can greatly decrease the risk of systemic toxicity. In general, a vasoconstrictor is added to the local anaesthetic preparations in order to delay systemic absorption. However, some of the vasoconstriction agents may be absorbed, occasionally to an extent sufficient to cause untoward reactions (Bloci & Covino 1981).

Liposomes have been used as drug carriers in an attempt to benefit drug therapy. Liposomal bupivacaine showed less systemic toxicity than bupivacaine solution after intravenous infusion in rabbits (Boogaerts et al 1993a). There is a report describing the plasma concentration of bupivacaine after plexus administration of bupivacaine liposomes and bupivacaine solution to rabbits (Boogaerts et al 1993b), but their anaesthetic effects were not studied or compared. Subcutaneous injection is one of the common routes of administration for local anaesthetic agents. However, the plasma concentration of bupivacaine after subcutaneous injection of its solution and liposomal formulation has not been reported. Since different administration routes may result in different plasma concentration profiles, those observed after intravenous (Boogaerts et al 1993a) and plexus administration (Boogaerts et al 1993b) may not be extrapolated to subcutaneous administration.

There are several types of liposomes, for example multilamellar vesicles, large unilamellar vesicles and small unilamellar vesicles. Multilamellar vesicles were

considered for the present study. They consist of several concentric spheres of lipid bilayers, which may act as multilayer membranes for controlled release. Although there are reports comparing the duration of local anaesthetic action between solution and liposomal formulations (Mashimo et al 1992; Grant et al 1994), their intensity of action has not been studied or compared. In a previous study, we observed that liposomal formulations could prolong the duration and enhance the intensity of the sensory block effect of bupivacaine (Yu et al 1998). However, the anaesthetic effect in combination with the pharmacokinetics of liposomal local anaesthetic agents has not been reported. The purpose of the present study was to investigate the pharmacodynamics and pharmacokinetics of a local anaesthetic agent in solution and in liposomal formulations after subcutaneous administration. Bupivacaine was used as a model drug. This study measured the efficacy in association with plasma concentrations, an index of systemic toxicity of a subcutaneously administered local anaesthetic agent, and specifically investigated how a novel method of drug delivery would affect the sensory block effect and the absorption of bupivacaine after subcutaneous injection.

Materials and Methods

Materials

Bupivacaine hydrochloride crystalline powder was a gift from Astra (Sweden). Cholesterol (CH), egg yolk phosphatidylcholine (PC) and phosphatidic acid (PA) were purchased from Sigma (St Louis, MO). Wistar rats were obtained from the Experimental Animal Center, College of Medicine, National Taiwan University. The animal study was carried out according to protocols approved by the Institutional Animal Care and Use Committee at the College of Medicine, National Taiwan University.

Preparation of liposomes

PC and CH were used as the liposome materials because they are non-toxic and non-immunogenic (Langer 1990). The liposome formulation contained PA to contribute anionic surface charge to the vesicle membrane in order to maximize the vesicle capacity (inner volume) and improve the encapsulation of bupivacaine hydrochloride (protonized bupivacaine) by electrostatic force. Bupivacaine liposomes were prepared by a mechanical shaking method (New 1990). A chloroform solution containing PC, CH and PA in a weight ratio of 1:0.4:0.1 was evaporated in a rotary evaporator under a stream of nitrogen gas to yield a thin lipid film, and then put in a vacuum desiccator overnight to ensure complete removal of traces of organic solvent. The dried lipid film was vortexed with 1.5% bupivacaine hydrochloride solution to produce multilamellar vesicles entrapping the drug solution, which were collected by centrifugation at 2500 *g* for 10 min. Blank liposomes were prepared by the same procedure, but using normal saline solution instead of bupivacaine solution. The size of liposomes was determined by a particle analyser (Coulter N4 Plus, Submicron Particle Sizer). The concentration of bupivacaine in liposomes was determined by a spectrophotometric method as follows. Bupivacaine liposomes (0.02 mL) were diluted with hydrochloric acid (0.1 M) to 5.0 mL and liposomal lipids were removed by three repeated extractions each with 0.2 mL methylene dichloride. Bupivacaine hydrochloride in the aqueous layer was measured by a spectrophotometer (Hitachi U-2000) set at 220 nm against a blank solution prepared by treating blank liposomes simultaneously. Drug concentrations were calculated using a linear calibration curve obtained from bupivacaine hydrochloride standard solutions (0.10–0.60 mg mL^{-1}). The coefficient of variation was less than 5%. The recovery of bupivacaine from liposomes was assessed to be complete. The liposomal pellets were suspended in distilled water to obtain a concentration in liposomes of 5 mg mL^{-1} encapsulated bupivacaine hydrochloride.

Tail-flick test (Grant et al 1993)

Wistar rats (230–250 g) were used for the tail-flick test. The experiment was performed as described previously (Yu et al 1998). The rat was fixed on a tail-flick test apparatus (Tail Flick Model DS20; Shinohara Electric Institute, Japan). The central portion of tail was exposed to heat produced by a projector lamp. A single control switch simultaneously activated the light and a timer, and the timer automatically stops when the exposed tail flicks. The time interval between switching on the light to the tail flick was recorded as the tail-flick latency. The tail-flick latency measured before drug treatment was defined as baseline. The intensity of the heat was adjusted to produce a baseline of between 5.0 and 7.0 s. A 15-s cut-off time was used to avoid thermal injury. Three measurements were made for each rat at each time point. Failure to flick the tail by this time was taken to indicate sensory block. The rats were divided into three groups: (i) the first group was treated with 0.2 mL bupivacaine solution containing 1 mg bupivacaine; (ii) the second group was treated with 0.2 mL bupivacaine liposomes encapsulating 1 mg bupivacaine; and (iii) the third group was treated with 0.2 mL blank liposomes. An insulin syringe (28-gauge needle attached to a 0.5 mL syringe) was used to inject the test solutions at the root of the tail on opposite sides of the midline (0.1 mL each side). The tail-flick test was started 15 min after drug administration, and the test was done every 15 min during the first 2 h, followed by hourly, and then every 15 min during the recovery period until the tail-flick latency declined to baseline. Each preparation was studied in five rats. The maximum possible effect (MPE) (Grant et al 1993) was calculated as follows:

$$\%\,\mathrm{MPE} = \frac{\text{tail-flick latency} - \text{baseline}}{\text{cut-off time} - \text{baseline}} \times 100$$

Pharmacokinetic study

Three groups of five rats each were studied. Two groups of rats were treated with bupivacaine liposomes and bupiva-

caine solution, respectively, exactly as for the tail-flick test. The third group received an intravenous bolus dose of 0.25 mg bupivacaine solution. Blood samples were collected at scheduled time points from a carotid artery cannula and immediately analysed for the plasma concentration of bupivacaine by high-performance liquid chromatography (HPLC) (Yu et al 1999). Briefly, 0.15 mL tetracaine hydrochloride solution (1 μg mL^{-1}) as an internal standard was added to a 0.15-mL plasma sample and alkalinized with 0.2 mL 1 M sodium hydroxide. The mixture was vortexed with 4 mL *n*-hexane for 2 min and then centrifuged (Sigma-202 MK, FRG) at 3000 rev min^{-1} at 4°C for 20 min. Then, 3 mL of the hexane phase was transferred into another test tube and evaporated to dryness under nitrogen flow at ambient temperature. The residue was dissolved in 0.15 mL of the mobile phase. A 50-μL aliquot was injected onto the HPLC system for analysis. The HPLC system consisted of a Waters Model 510 pump (Waters Association, Milford, MA, USA) equipped with a spectrophotometric detector (Linear UVIS 200) set at 205 nm, and a Waters 740 Data Module recorder. A reverse-phase analytical column, C_{18} (250 × 4 mm i.d., particle size 5 μm) (E. Merck, Darmstadt, Germany) was used. The mobile phase was a mixture of 10 mM phosphate buffer (pH 3.0) and acetonitrile (65/35, v/v) containing 0.3% diethylamine. The flow rate was 0.8 mL min^{-1}. The method provided > 90% accuracy and precision over the range 0.025 to 5 μg mL^{-1}.

Pharmacokinetic parameters were analysed by WinNonlin 1997 (SCI Software, Pharsight, Mountain View, CA). The cumulative relative fraction of systemic absorption was estimated by the method of Loo-Riegelman (Shargel & Yu 1993a) using plasma concentration data.

The Student's *t*-test was used to compare two groups. Pharmacodynamics, in terms of anaesthetic duration, MPE at each time-point, and overall MPE, between bupivacaine solution and bupivacaine liposomes were compared. Differences in pharmacokinetic parameters between the two routes of administration (i.v. vs s.c.) of bupivacaine solution, and between the two formulations (bupivacaine solution vs bupivacaine liposomes) by subcutaneous injection were compared. A value of $P < 0.05$ was considered significant for all comparisons.

Results

Bupivacaine liposomes

The bupivacaine liposomes were prepared by a conventional method for producing multilamellar vesicles (Lasic 1988). The average ± s.d. size of bupivacaine liposomes was 1.1 ± 0.6 μm, and the total lipid concentration was 25 mg mL^{-1}. The content of bupivacaine in liposome preparations was 5 mg mL^{-1}. The encapsulation ratio of bupivacaine was 0.2 mg (mg lipid)$^{-1}$. The pH of bupivacaine solution and bupivacaine liposomes was 6.35.

Tail-flick test

Blank liposomes did not show a sensory block effect in any of the treated rats. A sensory block effect of both bupivacaine liposomes and bupivacaine solution was observed in the initial tail-flick test (15 min after drug administration), showing MPE values of 97.5 ± 3.5% (mean ± s.d.) and 3.6 ± 7%, respectively (Figure 1). Bupivacaine liposomes achieved the MPE significantly ($P < 0.01$) faster than bupivacaine solution (100% at 30 min vs 47.6 ± 13.7 % at 45 min, respectively). The duration of the sensory block with bupivacaine liposomes (405–480 min) was 5-times longer than that with bupivacaine solution (75–90 min) (Table 1).

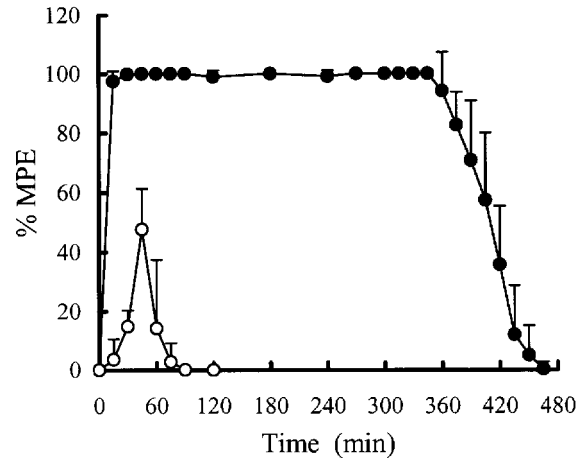

Figure 1 Duration and percentage maximum possible effect (% MPE) of bupivacaine solution (○) and bupivacaine liposomes (●) in the rat tail-flick test. Data are presented as mean ± s.d., n = 5. The % MPE was significantly different ($P < 0.01$) between bupivacaine liposomes and bupivacaine solution at each time point.

Table 1 Sensory block effect of bupivacaine solution and bupivacaine liposomes in rats.

	Bupivacaine solution	Bupivacaine liposomes	Blank liposomes
Onset time (min)	< 15	< 15	–
Maximum possible effect (%)	47.6 ± 13.7	100 ± 0**	0
Duration (min)	87 ± 7	447 ± 28**	0

Data are mean ± s.d., n = 5. **$P < 0.01$, significant difference between bupivacaine solution and bupivacaine liposomes.

Pharmacokinetic study

Intravenous injection of 1 mL bupivacaine solution (0.5 mg mL^{-1}) by slow push (approx. 0.2 mL min^{-1}) to three rats caused the sudden death of two rats before finishing the injection. Reducing the dose by half (1 mL diluted bupivacaine solution : 0.25 mg mL^{-1}) made the intravenous study possible. Plasma concentration–time profiles (Figure

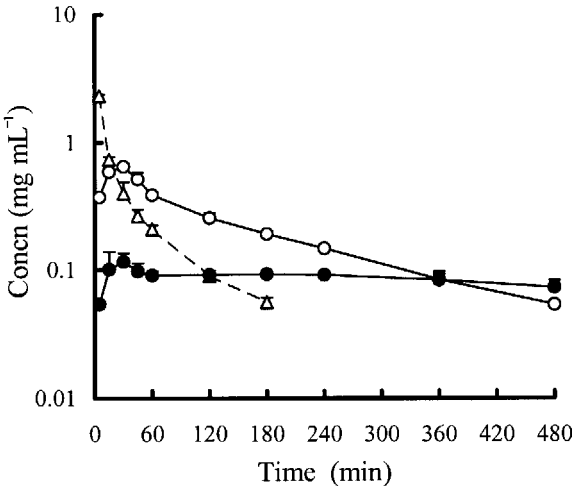

Figure 2 Mean plasma concentrations of bupivacaine in rats after intravenous injection of 0.25 mg bupivacaine solution (△), subcutaneous injection of 1 mg bupivacaine solution (○) or bupivacaine liposomes (●). Data are presented as mean±s.d., n = 5.

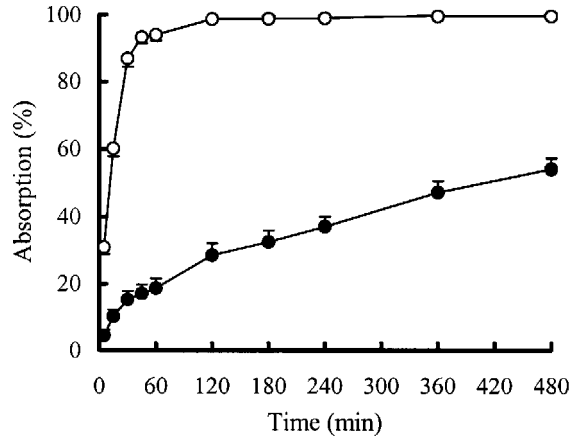

Figure 3 Systemic absorption–time curves of bupivacaine after subcutaneous injection of bupivacaine solution (○) and bupivacaine liposomes (●).

2) of bupivacaine were fitted to a two-compartment model for bupivacaine solution, and to a non-compartment model for bupivacaine liposomes. The pharmacokinetic parameters obtained are listed in Table 2. With bupivacaine solution, the systemic absorption after subcutaneous injection, in terms of dose fraction (F), was 41.7±4.2% ; the rate constants of the distribution phase (α) and elimination phase (β) were significantly smaller, and the mean residence time (MRT) was significantly longer after subcutaneous injection than after intravenous injection. However, the plasma clearance (CL) was not significantly different between the two administration routes.

The peak plasma concentration (C_{max}) after subcutaneous injection of bupivacaine solution was 5-fold that of bupivacaine liposomes (0.65±0.04 vs 0.12±0.02 μg mL^{-1}); both appeared at 30 min. The systemic absorption of bupivacaine solution after subcutaneous injection was rapid (absorption half-life, $t_{1/2}(k_{01})$ = 9.9±2.3 min);

Table 2 Pharmacokinetic parameters of bupivacaine in rats.

Parameter	Two-compartment model		Non-compartment model	
	Bupivacaine solution 0.25 mg, i.v.	Bupivacaine solution 1 mg, s.c.	Bupivacaine solution 1 mg, s.c.	Bupivacaine liposomes 1 mg, s.c.
A (μg mL^{-1})	4.48±0.31	6.12±4.55	–	–
B (μg mL^{-1})	0.55±0.16	0.40±0.11	–	–
A (10^{-3} min^{-1})	181±21	51±6[a]	–	–
β or Z (10^{-3} min^{-1})	14.7±3.1	3.7±1.2[a]	3.7±1.2	0.9±0.2[b]
k_{01} (10^{-3} min^{-1})		73±20	–	–
k_{10} (10^{-3} min^{-1})	81±4.4	12±1.7[a]	–	–
$t_{1/2}(\alpha)$ (min)	3.8±0.4	13.8±1.7[a]	–	–
$t_{1/2}(\beta)$ (min)	48.5±10.2	176.6±42.8[a]	176.6±42.8	745.5±149.1[b]
$t_{1/2}(k_{01})$ (min)		9.9±2.3	–	–
T_{max} (min)		21.1±0.4	30±0	30±0
C_{max} (μg mL^{-1})	5.04±0.45	0.63±0.05[a]	0.65±0.04	0.12±0.02[b]
AUC_{0-480} (min μg mL^{-1})	61.9±3.1	96.5±2.9[a]	96.5±2.9	42.0±2.2[b]
$AUC_{0-\infty}$ (min μg mL^{-1})	65.6±2.9	109.5±7.6[a]	109.5±7.6	120.6±9.9
F (%)		41.7	41.0	42.1
CL (mL min^{-1})	3.71±0.16	3.84±0.21	3.84±0.21	3.49±0.32
Vd_{ss} (mL)	219.9±15.2	791.8±138.0	–	–
MRT (min)	57.7±1.5	206.2±37.3[a]	206.2±37.3	1095.0±198.0

Data are mean±s.d., n = 5. [a]Significant difference between intravenous and subcutaneous solutions. [b]Significant difference between subcutaneous solution and liposomal formulation.

however, the plasma elimination was relatively slow (elimination phase half-life, $t_{1/2}(\beta)$ = 176.6±42.8 min). The pharmacokinetics of bupivacaine were greatly altered by entrapping bupivacaine in liposomes. After subcutaneous injection, bupivacaine liposomes showed a small peak followed by a steady plasma level of bupivacaine (0.091±0.004 μg mL^{-1}) over several hours, and the area under the plasma elimination curve up to 480 min (AUC_{0-480}) was less than half that resulting from bupivacaine solution (42.0±2.2 vs 96.5±2.9 min μg mL^{-1}). The systemic absorption curves of bupivacaine after subcutaneous injection are shown in Figure 3. Absorption of bupivacaine from bupivacaine solution was nearly complete (> 93%) in 45 min, but that from bupivacaine liposomes was only 17% complete in 45 min and less than 54% complete in 480 min.

Pharmacokinetics and pharmacodynamics

In rats treated with bupivacaine solution, the C_{max} of bupivacaine appeared earlier than the MPE, and at the time of the MPE, the systemic absorption was nearly complete (Figure 3). The MPE then declined to baseline at 90 min when the plasma concentration of bupivacaine was still more than half the C_{max}. On the other hand, bupivacaine liposomes achieved the MPE in the initial tail-flick test (15 min) with only 10% systemic absorption. The 100% MPE plateau lasted to 360 min, with only 47% cumulative systemic absorption (Figure 3), and with a low and constant plasma concentration (0.091±0.004 μg mL^{-1}) during this period.

Discussion

The concentration of bupivacaine used in this experiment (0.5%) is the usual clinical concentration for peripheral nerve block. It is very toxic by intravenous injection as observed in this study; a dose of 1 mL 0.05% aqueous solution per rat (equivalent to half the dose for the tail-flick test) at an infusion rate of 0.2 mL min^{-1} (= 0.1 mg min^{-1}) resulted in the death of rats before finishing the infusion.

In theory, during the initial period after injection, the concentration of readily available bupivacaine in liposomes should be much less than that in solution to achieve nerve penetration, and therefore the onset of sensory block with bupivacaine liposomes should be slower, and the intensity lower, than that with bupivacaine solution. However, in a previous study (Yu et al 1998) and in the present study, we found that bupivacaine liposomes revealed a significantly higher MPE (approx. 27-fold) than bupivacaine solution in the initial tail-flick test (97.5±3.5 vs 3.6±7%). Our results agree with a report that the onset of the anaesthetic effect of lidocaine liposomes was faster than that of lidocaine solution after extradural injection in dogs (Mashimo et al 1992). These results are rational based on the pharmacology of bupivacaine. Quaternary analogues of local anaesthetics act in the charged form to block nerve conduction from the inner surface of the nerve cell membrane, but are relatively ineffective outside the cell membrane (Strichartz & Ritchie 1987). Therefore, they must first cross the nerve cell membrane into the cells before they can exert a blocking action. However, quaternary analogues in the charged form cannot permeate through the cell membrane. At physiological pH, only about 10% of bupivacaine (pK_a = 8.09) (Budavari 1996) is in the non-charged form that can permeate through the cell membrane and then equilibrate to the charged form inside the nerve cell to exert its action. Alkalinization of bupivacaine hydrochloride solution can hasten the onset time of peripheral nerve block by increasing the proportion of the membrane-permeable unionized free base (Coventry & Todd 1989). Our results suggest that liposomes may facilitate the transfer of charged bupivacaine into nerve cells and exert an instant blocking action from the inner surface of the cell membrane. Phospholipids possess the property of surfactant, which is thought to penetrate into the intercellular lipid bilayers, thereby reducing the crystallinity of the intercellular lipid bilayers and thus increasing the permeability of these bilayers (Kadir et al 1987). Membrane-impermeable hydrophilic molecules can be transferred into cells by encapsulation in liposomes (Yu & Lin 2000). Possibly by the same mechanism, the charged bupivacaine in liposomes may be transferred into nerve cells and may thereby omit the lag time for equilibration between charged and uncharged forms before and after membrane permeation, and rapidly provide an effective intracellular concentration of charged bupivacaine. Based on this mechanism of action, it can be speculated from our results that liposomes may facilitate the transmembrane activity of quaternary analogues of local anaesthetic agents into nerve cells with the result of rapid onset and enhanced action. However, such speculation needs to be verified by further studies.

As expected, the plasma concentration–time profiles of bupivacaine resulting from subcutaneous injection of bupivacaine solution and bupivacaine liposomes were different. Bupivacaine liposomes gave a significantly lower plasma concentration, which was more or less constant for several hours. These results do not agree with the results of a study in rabbits treated by plexus administration (Boogaerts et al 1993b), which showed that liposomal bupivacaine resulted in slower elevation of the plasma concentration to a higher C_{max} than bupivacaine solution. The disagreement may be attributed to the different administration routes (plexus vs s.c.). In addition, in the study by Boogaerts et al (1993b), free bupivacaine base was intercalated in liposomal lipid bilayers and compared with bupivacaine hydrochloride solution. The physical property of the active ingredient (free base vs salt form), and the pH of vehicles (pH 8.1 for liposomes vs slightly acidic for solution) were different in their study, which may not only affect the pharmacokinetics, but also the local anaesthetic response (Fernando & Jones 1991).

The $AUC_{0-\infty}$ of bupivacaine after subcutaneous injection of bupivacaine solution and bupivacaine liposomes was almost equivalent. However, the toxicity of bupivacaine was closely related to the plasma concentration or the shape of the AUC, but not the value of AUC. Bupivacaine liposomes suppressed the plasma level of bupivacaine to a

low (safe) and steady level throughout the whole anaesthetic period.

The latency of the MPE (45 min) behind the C_{max} resulting from subcutaneous injection of bupivacaine solution implies its wide diffusion area over the capillary bed so that total systemic absorption is more rapid than accumulation of sufficient drug concentration inside the nerve. The relatively rapid absorption of bupivacaine into systemic circulation decreases the amount of locally available drug, resulting in insufficient drug to produce a pharmacological effect. This mechanism may cause the MPE of bupivacaine solution to begin to decline (after 45 min) as soon as most of the drug ($>90\%$; Figure 3) has been absorbed systemically. Subcutaneous absorption of bupivacaine solution was almost complete within 60 min (Figure 3); at that time, the sensory block effect had declined to less than 10% MPE, and the plasma concentration was still three-fifths the C_{max}. In such conditions, additional doses of bupivacaine solution for prolonging local anaesthesia may elevate plasma concentrations to toxic levels. The toxic threshold for bupivacaine plasma concentrations is considered to be 2 μg mL^{-1} (Tucker 1986).

With bupivacaine liposomes, the sensory block effect lasted for longer than 7 h, with a steady low plasma concentration. A progressive and sustained release of bupivacaine from multilamellar vesicles (Malinovsky et al 1999) could afford a local interstitial constant level of free bupivacaine, which may ensure prolonged duration of local anaesthesia, and suppress the peak plasma concentration of the encapsulated drug. This result suggests that liposomes could localize the encapsulated bupivacaine at the injection site, restrict the systemic absorption area and release their content slowly.

The mean absorption time (MAT) of bupivacaine solution, and the mean dissolution time (MDT) of bupivacaine liposomes after subcutaneous injection could be estimated by the following equations (Shargel & Yu 1993b).

$$\text{MAT} = \text{MRT}_{\text{s.c.}}(\text{bupivacaine solution/liposomes}) - \text{MRT}_{\text{i.v.}}(\text{bupivacaine solution})$$

$$\text{MDT} = \text{MRT}_{\text{s.c.}}(\text{bupivacaine liposomes}) - \text{MRT}_{\text{s.c.}}(\text{bupivacaine solution})$$

The subcutaneous MAT of bupivacaine solution and liposomes thus obtained was 148.5 and 1037.3 min, respectively, which means that the systemic absorption of liposomal bupivacaine is much slower than that of bupivacaine solution. The MDT, which reflects the time for liposomal bupivacaine to release in-vivo, was 888.8 min. The long MDT represents a sustained release of bupivacaine from liposomes in-vivo.

The larger Vd_{ss} resulting after subcutaneous injection than after intravenous injection of bupivacaine solution may be attributed to a sequestration of bupivacaine by cutaneous fat and tissues around the injection site. Such sequestration may also be responsible for the low F (41.8%) after subcutaneous injection. According to the relationship of $CL = k_{10} \times V_{dss}$, the values of Vd_{ss} and k_{10} counterbalanced mutually, resulting in no significant difference in CL between intravenous and subcutaneous injections.

In conclusion, encapsulation of bupivacaine solution in liposomes could greatly increase the intensity and duration of the sensory block effect, and decrease the systemic toxicity by suppressing the systemic absorption rate. The dose required to produce the same level of local anaesthesia with a liposome formulation might be much less than with the solution form. These qualities make it possible for liposomal formulations of bupivacaine to provide a safe and effective ultra-long anaesthesia with a single usual dose. Liposomes are preferable formulations for local anaesthetic agents.

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