Prolongation of Nerve and Epidural Anesthetic Blockade by Bupivacaine in a Lipid Emulsion

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We assessed the effect of a lipid emulsion of bupivacaine on prolonging peripheral nerve and epidural anesthetic blockade in the rat. The intensity and duration of motor and sensory blockade produced by a single injection of aqueous solution (BPV-as) and lipid emulsion (BPV-em) preparations of 0.5% bupivacaine were evaluated by electrophysiological methods. Both preparations induced complete, reversible motor and sensory blockade after injection. The latency time to the maximal blockade and the duration of anesthetic blockade were more prolonged for BPV-em than for BPV-as. The increase in duration of maximal blockade was 1.4 times for nerve and 1.3 times for epidural anesthesia. Histological evaluation of spinal roots and spinal cord sections did not show any abnormalities or differences between animals injected with BPV-as and those injected with BPV-em. Pharmacokinetic studies showed lower plasma peak concentration and a longer elimination half-life for BPV-em than for BPV-as. Thus, BPV-em prolongs the effects of local anesthetics, allows a similar degree of blockade, and reduces the systems toxic effects of anesthetics compared with BPV-as. **Implications:** We assessed a lipid emulsion containing bupivacaine for peripheral nerve and epidural anesthetic blockade in the rat. The emulsion allowed a complete blockade, while increasing the duration of the anesthetic effect (by 30%–40%), compared with the standard bupivacaine aqueous solution.

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Local anesthetics are widely used for providing regional anesthesia, postoperative analgesia, and treatment of chronic pain conditions. However, the relatively short duration of action, when available local anesthetics are administered as a single injection, limits the applicability to procedures lasting 4–6 h. Increasing the dose of the anesthetic is not recommended because it may result in severe cardiovascular and respiratory complications (1–3), toxicity to neural tissues (4–6), and neurological sequelae (7). Longer lasting effects are usually achieved by continuous or repeated administration via indwelling catheters for regional, epidural, or spinal anesthesia. However, complications associated with catheterization, including intravascular injection, migration of the catheter, catheter breakage, cauda equina syndrome (8), inflammatory changes in the spinal leptomeninges (9,10), and fibrosis at the catheter tip (11), have been reported. Prolongation of the anesthetic effect by a single administration of slow-releasing preparations could expand the applications of local anesthetics. They can be used to decrease the risk of side effects as a result of excessively high plasma concentrations, and to avoid catheterization. Different lipid carrier preparations have been assayed for these purposes. Liposomes prolong the nerve-blocking effect of lidocaine and bupivacaine (12,13), and transfersomes enhance the analgesic effects of lidocaine when applied topically or subcutaneously (14). A lipid solution of iophendylate also produces a significant prolongation of anesthetic effects in spinal and epidural anesthesia (15–17). Other strategies in the search for prolonged anesthetic or analgesic effects include research on synergistic interactions of coadministered anesthetics that exert variable effects either additive or deficient depending on the dose and combination used (18); on quaternary ammonium derivatives of local anesthetics that provide ultralong blockades but have neurolytic effects causing axonal degeneration (19); and on sustained release of local anesthetics from surgically implanted biodegradable polymers (20). We evaluated

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the usefulness of a lipid emulsion preparation of bupivacaine in prolonging peripheral nerve and epidural anesthetic blockade in the rat using neurophysiological tests that provide quantitative assessment of nerve conduction blockade.

Methods

Female Sprague-Dawley rats, weighing 250–300 g, were distributed in four groups of 8–10 animals each according to the solution administered for nerve and epidural anesthesia. The animals were maintained under standard conditions, with access to food and water *ad libitum*. We complied with the National Research Council's *Guide for the Care and Use of Laboratory Animals* throughout the study, and the study was approved by the ethics committee of our institution.

Bupivacaine was administered either as an aqueous solution of 0.5% bupivacaine hydrochloride in saline solution (BPV-as) or as a lipid emulsion (BPV-em). The BPV-em consisted of soy bean oil 10 g, Miglyol® (triglycerides of C8-10 fatty acids, Hüls AG, Marl, Germany) 10 g, egg lecithin 1.2 g, glycerol 2.5 g, natrium oleate 0.03 g, bupivacaine base 0.45 g, and water to 100 mL. Preparation of the emulsion started by dissolving bupivacaine base in the mixture of soy bean oil and Miglyol®; subsequently, the oily phase was mixed with the aqueous phase and emulsified by using a high-pressure homogenizer (APV Gaulin, Houten, The Netherlands). The oil droplet size was <1 μm in diameter, and the percentage of bupivacaine encapsulated was more than 99%, with a shelf-life of 18 mo at 25°C. The emulsion was heat-sterilized and stored in aliquots at 4°C. The physicochemical characteristics of the emulsion are those of fat emulsions intended for IV administration for parenteral nutrition. Bupivacaine preparations were provided by B. Braun Medical, Rubí, Spain.

All procedures were performed under general anesthesia with pentobarbital (40 mg/kg intraperitoneal, plus additional doses if required). The degree of general anesthesia was monitored by eliciting the corneal reflex. Once anesthetized, the back and the outer aspect of the hindlimbs were shaved and disinfected with povidone iodine, and the animal was placed on a thermostat controlled flat plate to maintain skin temperature >32°C.

Sciatic nerve blockade was performed by a single transcutaneous injection of 0.2 mL of bupivacaine solution in the popliteal space of the hindlimb. The injection point is equidistant between the knee and the L6 spinal process, and it corresponds to the space around the sciatic nerve above its trifurcation. Epidural injection was performed by a transcutaneous puncture with a 27-gauge needle at the L5-6 intervertebral space by inserting the needle along the right side of the spinous apophysis until the rounded tip entered the vertebral foramen. After aspiration for evidence of blood or cerebrospinal fluid proved negative, 0.2 mL of the bupivacaine preparation was slowly injected over 20 s (21). In preliminary assays, we established the accuracy of the needle location for nerve and epidural blockades by injecting a methylene blue solution and locating it after dissection of the corresponding area.

We evaluated the sciatic nerve blockade using nerve conduction studies of motor and sensory nerve fibers. The sciatic nerve was stimulated by two needle electrodes inserted at the sciatic notch, applying single pulses of 0.1 ms and voltage necessary to obtain a supramaximal response. Compound muscle action potentials, evoked by stimulation of motor nerve fibers, were recorded from the plantar muscles with monopolar needles; at the same time, compound nerve action potentials, elicited by stimulation of large sensory fibers, were recorded from the digital nerves of the third toe by using small needle electrodes near the nerve (22). Compound action potentials were amplified and displayed on a digital oscilloscope under appropriate settings to measure the amplitude of the negative peak and latency to the onset.

Neurophysiological techniques designed to quantitatively assess the function of lumbar spinal reflex arches were used to evaluate the epidural blockade (21). The myotatic stretch reflex was evaluated by the H wave, which was recorded by using motor nerve conduction tests set as above. The amplitude and the latency of the H wave were measured. The withdrawal reflex responses were elicited by electrical pulses (supramaximal voltage, 0.5 ms) applied via monopolar needles adjacent to the tibial nerve at the ankle. The contralateral extensor reflex was evaluated by recording the motor response from the left quadriceps muscle with monopolar needles. The active IM electrode was positioned to record the evoked volley of motor unit action potentials at their greatest amplitude. We measured the maximal amplitude, latency to onset, and duration of the volley of motor unit action potentials.

Nociceptive responses were elicited by electrical shocks (pulses of 100 V, 0.1 ms) applied to the plantar surface of the hind paw by means of a bipolar metal electrode with conducting gel at the tips (21). The normal response was a reflex muscle contraction, most evident at the lumbar and abdominal muscles. At each test interval, the response to two consecutive stimuli was scored on a 3-point scale, where 2=response was brisk and bilateral, 1=light or unilateral, or 0=absent.

All tests were performed in duplicate before the anesthetic injection to obtain individual control values; at intervals of 1, 3, 5, 7, and 10 min; and each 5 min thereafter until complete recovery. At each time, the voltage of stimuli was maintained 25% above that

which gave a maximal response before injection, and stimuli were given three times to obtain the best response. Recorded values for each interval after injection were normalized as the percentage of the control values for each animal and plotted against time as the mean of all animals in each group. In addition, the following variables were calculated: onset of the anesthetic blockade, latency time to maximal blockade, amplitude and duration of the smallest response (maximal blockade), time and amplitude of the maximal recovery response, and total duration of the anesthetic blockade. Statistical comparisons between groups were made by using the Mann-Whitney *U*-test and were considered significant if $P < 0.05$.

To assess possible damage to the nervous system, 12 rats were studied 1 week after epidural injection: 4 with saline solution, 4 with 0.5% BPV-as, and 4 with 0.5% BPV-em. Animals were anesthetized and transcardially perfused with 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 5% sucrose. Tissue samples were dissected at the L1-2 and L4-5 levels and immersed in the same fixative solution for 4 h. After decalcification in 10% formic acid and 4% paraformaldehyde, samples were rinsed in tap water and 0.05 M phosphate buffered solution (pH 7.4), dehydrated and embedded in paraffin. Sections (8 μm) were stained with hematoxylin-eosin and Klüver-Barrera stains. Stained sections of spinal cord, lumbosacral roots, dorsal root ganglia, and meningeal membranes were inspected under light microscopy.

Sciatic nerve blockade was performed in two groups of 36 rats: one group received BPV-as and the other received BPV-em. The effectiveness of the blockade was assessed by pinching the toes with forceps every 5 min after injection. Blood samples were obtained by decapitation of three anesthetized rats at each time point between 5 and 300 min after blockade, placed in heparinized tubes, and centrifuged. The plasma was withdrawn and stored at −20°C until analyzed. The plasma concentration of bupivacaine was determined using high-performance liquid chromatography as previously described (23). Plasma bupivacaine concentrations were analyzed by a model-dependent analysis. Results were fitted to a one- and two-compartment open model using weighted and unweighted data, determined by using a nonlinear least-squares regression program. Pharmacokinetic variables after the administration of BPV-as and BPV-em were compared by using Wilcoxon's signed rank test and were considered significant if $P < 0.05$.

Results

Both BPV-as and BPV-em preparations were effective in inducing complete (amplitude of the action potentials = 0) motor and sensory nerve conduction block that reversed to normal values after variable postinjection times (Fig. 1). Blockade was similarly effective for sensory and motor large nerve fibers. With BPV-as, the blockade started 1 min after injection, and the maximal effect lasted from approximately 15 min to 124 ± 14 min (mean ± SD) and 158 ± 17 min for motor and sensory fibers, respectively, whereas progressive recovery was observed during the following 120 min. With BPV-em, the anesthetic effect started equally fast—by 2 min—but a complete blockade was achieved later—by 34 ± 4 and 20 ± 3 min ($P < 0.05$ versus BPV-as)—and lasted up to 166 ± 28 and 209 ± 29 min for motor and sensory fibers, respectively. The latency time to the maximal blockade and the total time until complete recovery were significantly prolonged for BPV-em compared with BPV-as (Table 1).

Rats treated with an injection of saline solution or BPV-em showed no nerve blockade during serial testing for at least 45 min.

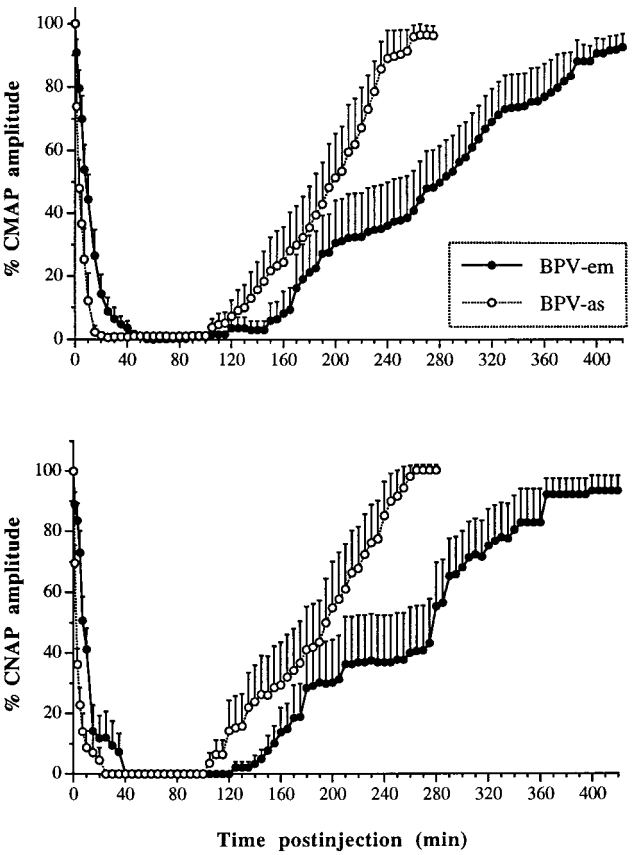

Figure 1. Changes over time after sciatic nerve blockade with bupivacaine in aqueous solution (BPV-as) or in lipid emulsion (BPV-em) on (A) the amplitude of the plantar compound muscle action potential (CMAP) (motor blockade) and (B) the amplitude of the digital compound nerve action potential (CNAP) (sensory blockade). Values are the mean percentage with respect to the preinjection control response for each rat.

Table 1. Comparison of Sciatic Nerve Blockade with Bupivacaine in Aqueous Solution (BPV-as) and in Lipid Emulsion (BPV-em)

	Motor blockade		Sensory blockade	
	BPV-as ($n = 9$)	BPV-em ($n = 8$)	BPV-as ($n = 9$)	BPV-em ($n = 8$)
Onset of block (min)	1 ± 0	2 ± 1	1 ± 0	3 ± 1
Latency to maximal block (min)	19 ± 4	34 ± 4*	12 ± 2	20 ± 3*
Degree of block (%)[a]	1 ± 1	0 ± 0	0 ± 0	0 ± 0
Duration of maximal block (min)	124 ± 14	166 ± 28	158 ± 17	209 ± 29
Time of complete recovery (min)	217 ± 10	309 ± 29*	213 ± 14	290 ± 29*
Degree of recovery (%)[a]	97 ± 3	94 ± 5	100 ± 2	94 ± 5

Values are mean ± SD.
* $P < 0.05$ with respect to BPV-as.
[a] Percentage of control values.

Both preparations of bupivacaine completely abolished the spinal reflex responses tested (Fig. 2). The temporal evolution of the three responses followed a similar course. With BPV-as, the anesthetic effect started within the first minute, and complete blockade lasted from 3 ± 1, 3 ± 1, and 2 ± 0 min to 47 ± 6, 47 ± 5, and 53 ± 8 min postinjection for the H reflex, the extensor reflex, and the nociceptive response respectively. All rats showed complete recovery of the responses by 100 ± 8, 95 ± 8, and 72 ± 97 min. With BPV-em, the blockade achieved maximal effect slightly later, but it was of significantly longer duration, lasting from 8 ± 2, 4 ± 1, and 5 ± 2 min to 70 ± 7, 80 ± 5, and 86 ± 5 min postinjection for the three responses, respectively, and complete recovery was achieved by 123 ± 8, 121 ± 6, and 117 ± 8 min postinjection. The duration of the maximal blockade, as well as the time to maximal recovery, was significantly longer for BPV-em than for BPV-as (Table 2).

All rats used for nerve or epidural blockade recovered after general anesthesia and showed no signs of disability, secondary effects, or infections.

The histological study of spinal cord sections showed no differences between animals injected with saline and those injected with bupivacaine formulations. In no case did the neuron bodies of anterior and posterior horns and ganglia show nuclear or cytoplasmic abnormalities. The spinal white matter and spinal roots presented a normal axonal pattern and myelin sheath appearance, and glial cells showed no abnormalities (Fig. 3). Meningeal covers had no remarkable changes except for a mild leukocyte infiltration in two animals treated with BPV-em.

The concentration-time curves of bupivacaine after the administration of both preparations were best fitted to two-exponential equations with a first-order input and a first-order elimination (Fig. 4). For BPV-as, the plasma concentration of bupivacaine showed a peak (Cmax) approximately 20 min after injection and was below the levels of detection after 120 min. After BPV-em administration, the bupivacaine plasma

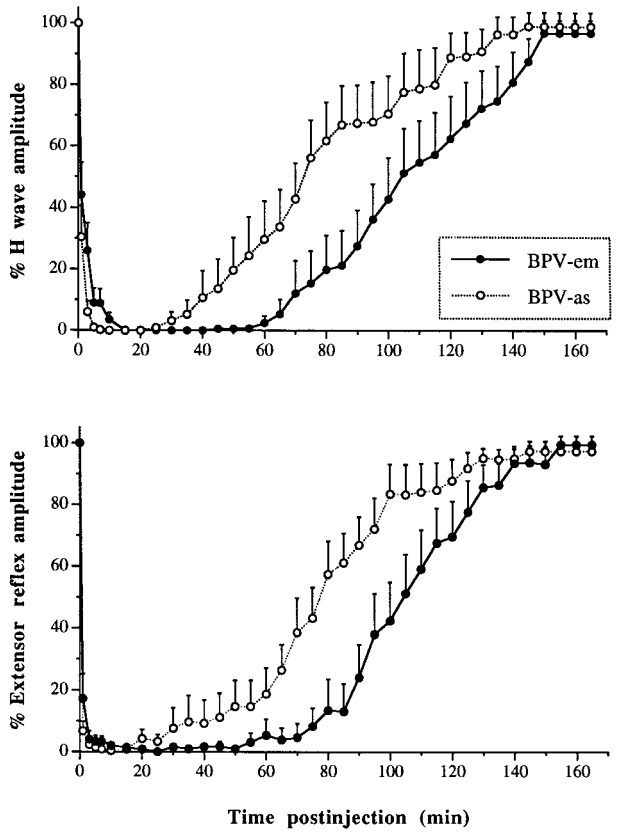

Figure 2. Changes over time after an epidural injection of bupivacaine in aqueous solution (BPV-as) or in lipid emulsion (BPV-em) on (A) the amplitude of the H wave and (B) the maximal amplitude the motor unit action potentials (MUAPs) of the extensor reflex. Values are the mean percentage with respect to the preinjection control response for each rat.

Cmax was lower and found significantly later, at 60 min postinjection, and bupivacaine was detected until 240 min. Table 3 shows the mean values of pharmacokinetic variables of the bupivacaine plasma concentration curves of the two groups. The absorption phase ($t1/2_{01}$) and the elimination phase ($t1/2_{10}$) half-lives were significantly longer for BPV-em than for BPV-as.

Table 2. Comparison of Epidural Blockade with Bupivacaine in Aqueous Solution (BPV-as) and in Lipid Emulsion (BPV-em)

	H wave		Extensor reflex		Nociceptive response	
	BPV-as (n = 10)	BPV-em (n = 10)	BPV-as (n = 10)	BPV-em (n = 10)	BPV-as (n = 10)	BPV-em (n = 10)
Onset of block (min)	1 ± 0	2 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Latency to maximal block (min)	3 ± 1	8 ± 2*	3 ± 1	4 ± 1	2 ± 0	5 ± 2
Degree of block (%)[a]	0 ± 0	0 ± 0	1 ± 1	1 ± 1	5 ± 4	0 ± 0
Duration of maximal block (min)	47 ± 6	70 ± 7*	47 ± 5	80 ± 5*	53 ± 8	86 ± 5*
Time of complete recovery (min)	100 ± 8	123 ± 8*	95 ± 8	122 ± 6*	72 ± 9	117 ± 8*
Degree of recovery (%)[a]	99 ± 5	97 ± 5	98 ± 3	100 ± 3	100 ± 0	100 ± 0

Values are mean ± SD.
* $P < 0.05$ with respect to BPV-as.
[a] Percentage of control values.

Discussion

Our results indicate that BPV-em prolongs the duration of peripheral nerve and epidural blockades while maintaining the intensity of its effect. Nerve conduction studies provide a classical quantitative assessment of peripheral nerve blockade. By simultaneous evaluation of motor and sensory nerve fibers conduction, we found that, after sciatic nerve block in the rat, the prolongation of the anesthetic blockade by the emulsion preparation was of the same order for both type of fibers. During epidural anesthesia, H-wave responses may be abolished by conduction block of either sensory or motor large fibers traveling within dorsal or ventral spinal roots, respectively, whereas abolition of the extensor reflex and the nociceptive response elicited by electrical stimulation of the tibial nerve depends on blockade of small size afferents related to nociceptors (21). The three responses evaluated followed a similar evolution, although BPV-em produced more prolonged (approximately 40%) maximal blockade of small afferents than that of large fibers producing the H wave, with respect to BPV-as. These differences may be explained by the anatomical distribution of nerve afferents in the dorsal roots at the entry zone into the spinal cord, with small fibers located more superficially, thereby being more accessible to the anesthetic (24).

The mechanism for prolonging the local anesthetic effect is based on the differential solubility of the ionized and free base forms of the drug in aqueous and lipid solutions (16,17). Because the base form of most local anesthetics is insoluble in water but soluble in lipids, the anesthetic remains retained in the lipid emulsion after injection and is slowly released from it after ionization that is limited to the molecules that reach the surface between the lipid and the aqueous milieu. Three kinetic processes determine the local anesthetic concentration and effect on the nervous tissue: diffusion of the drug from the injected solution into the extracellular fluid, uptake by the nerve fibers,

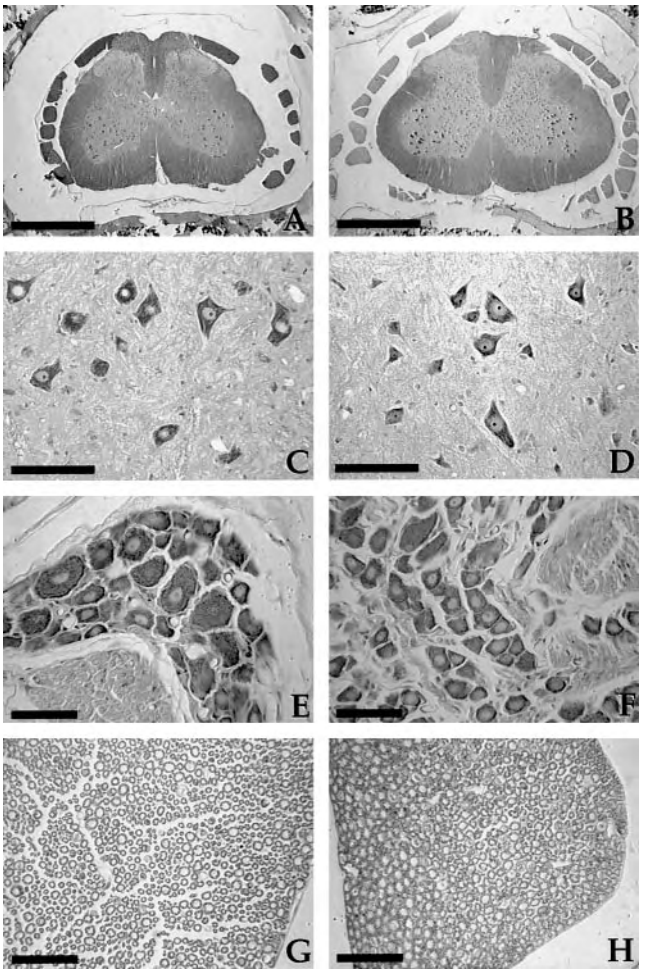

Figure 3. Histological sections from animals injected with bupivacaine in aqueous solution (A, C, E, G) and in lipid emulsion (B, D, F, H). A and B, Transverse sections of the spinal cords at the L1-2 level at low magnification. No histopathological changes are observed in white and gray matter. Bars correspond to 1 mm. C and D, The anterior horn. No changes in neurons and glial cells were found. Bars correspond to 100 μm. E and F, Sections taken at the dorsal root ganglia; ganglionar neurons have normal appearance. Bars correspond to 50 μm. G and H, Transverse sections of spinal roots with normal axonal pattern and without myelin abnormalities. Bars correspond to 50 μm. Klüver-Barrera–stained sections.

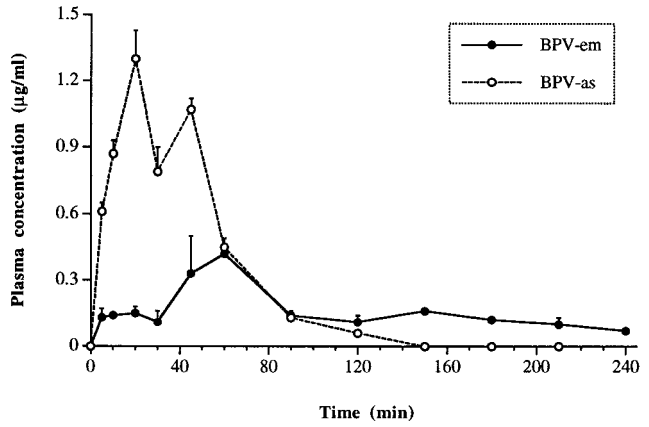

Figure 4. Bupivacaine plasma concentrations over time after the administration of bupivacaine in aqueous solution (BPV-as) and in lipid emulsion (BPV-em) for sciatic nerve blockade in rats. Values are mean ± SD.

Table 3. Pharmacokinetic Variables Corresponding to the Analysis of Plasma Concentration Curves After a Single Injection of a 4 mg/kg Dose of Bupivacaine in Aqueous Solution (BPV-as) and in Lipid Emulsion (BPV-em)

	BPV-as	BPV-em
K01 (min^{-1})	0.11 ± 0.04	0.03 ± 0.00*
K10 (min^{-1})	0.036 ± 0.007	0.006 ± 0.002*
AUC ($\mu g \cdot min/mL \cdot kg$)	67.4 ± 5.7	42.2 ± 2.2*
$t½_{01}$ (min)	7.3 ± 2.4	23.5 ± 3.6*
$t½_{10}$ (min)	19.7 ± 4.2	119.9 ± 26.6*
MRT (min)	41.7 ± 3.9	194.2 ± 36.7*
tmax (min)	16.7 ± 4.7	55.0 ± 7.1*
Cmax ($\mu g/mL$)	1.4 ± 0.1	0.5 ± 0.0*

Values are mean ± SD.

K01 = absorption rate constant, K10 = elimination rate constant, AUC = area under the plasma concentration-time curve, $t½_{01}$ = absorption phase half-life, $t½_{10}$ = elimination phase half-life, MRT = mean residence time, tmax = time of peak plasma concentration, Cmax = peak plasma concentration.

* $P < 0.05$ with respect to BPV-as.

and elimination to the blood flow. The first two processes determine the onset, spread, and intensity of the blockade, whereas the third influences the duration of the action (16). The pharmacokinetic results indicated that BPV-em had a slower absorption rate than BPV-as reaching peak plasma concentrations approximately 30% lower and significantly later. Bupivacaine was detectable in plasma samples up to 240 min after its administration in emulsion but only to 120 min in aqueous solution. As the components of the lipid emulsion used do not modify the catabolism of bupivacaine, the slower rate of elimination can be attributed to its delayed absorption from the emulsion. Therefore, the lipid emulsion allows for a longer presence of bupivacaine at lower peak plasma levels, thus reducing the risk of systemic toxicity. The prolonged presence of bupivacaine at the injection site was related to an increase in duration of 1.4 times for nerve blockade and 1.3 times for epidural blockade. Despite the slower absorption, the concentration of the local anesthetic available at neural tissues was high enough to produce a complete blockade of the same intensity as that achieved with the aqueous solution. In fact, Popitz-Bergez et al. (25) showed that, during *in vivo* peripheral nerve block, only 1.6% of the injected local anesthetic penetrates into the nerve during the period of full blockade.

Liposomes have been effectively used as slow-releasing carriers for local anesthetics. Mashimo et al. (12) found 3 times longer duration of epidural blockade in dogs with 2% lidocaine enclosed in unilamellar liposomes than with aqueous solution. Grant et al. (13) also reported longer duration of sensory block in the mouse nerve and lower systemic toxicity by administering 1% bupivacaine in liposomes. Encapsulation in liposomes led to longer time to peak concentration and longer duration of plasma concentrations of lidocaine (12). However, the large-scale manufacture of liposome products presents a variety of challenges, including particle size distribution, drug entrapment consistency, sterilization, stability during storage and removal of solvents and detergent. On the contrary, parenteral products containing emulsified vegetable oils have been in clinical use for nearly 30 years, and emulsions containing water-insoluble drugs (as propofol, etomidate, diazepam) are already marketed. There are well established technologies for their large-scale production, and they have satisfactory shelf life and physical properties.

Langerman et al. (15–17) reported that local anesthetics carried in a lipid solution of iophendylate significantly increased (2–3 times) the duration of spinal and epidural anesthesia in the rabbit. However, those lipid solution preparations caused a lower intensity of motor blockade than their corresponding aqueous solutions. Tetracaine was more effective than lidocaine and procaine, both in intensity and duration of blockade, mostly because of the higher partition coefficient and lipophilicity of the former. Bupivacaine has a high partition coefficient and liposolubility (26), a fast onset, and long-acting effect, thus being a suitable anesthetic for inclusion in lipid carrier preparations. Comparisons of our results with those of Langerman et al. (17) are difficult of because differences in species used, method of injection (transcutaneous puncture versus implanted catheter), local anesthetic assayed (bupivacaine versus tetracaine), and methods of assessment (neurophysiological tests versus semiquantitative Bromage scale). The advantage of the lipid emulsion we evaluated is that it prolongs the duration of the anesthetic blockade—although to a lower ratio than described for iophendylate solution—but maintains the intensity of the blockade with respect to the aqueous solution. In addition, aseptic arachnoiditis was reported to occur after the intrathecal administration of iophendylate (16). No histological abnormalities in the

spinal roots and spinal cord at the site of injection were found with BPV-em. Thus, the lipid emulsion represents an adequate slow-releasing carrier to prolong the effects of local anesthetics in regional anesthesia.

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