Sciatic Nerve Blockade with Lipid-Protein-Sugar Particles Containing Bupivacaine

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Purpose. To assess the efficacy of lipid-protein-sugar particles (LPSPs) in providing prolonged duration local anesthesia by percutaneous injection.

Methods. Bupivacaine-containing LPSPs were characterized and optimized *in vitro.* Male Sprague-Dawley rats were given sciatic nerve blocks with bupivacaine-containing LPSPs. Sensory and motor nerve blockade were measured in the hindpaw, as were contralateral functional deficits (a measure of systemic drug distribution). Poly(lactic-co-glycolic) acid (PLGA) microspheres were used as a reference.

Results. 10% (w/w) bupivacaine LPSPs (60% dipalmitoylphosphatidylcholine) were 4.4 ± 0.39 μm in diameter, with a tap density of 0.11 ± 0.04 g/ml. These LPSPs and 50% (w/w) PLGA microspheres had comparable durations of sensory blockade (468 ± 210 min vs. 706 ± 344 min, $p = 0.08$), although the LPSPs produced a much lesser duration of motor blockade (508 ± 258 min vs. 1062 ± 456 min, $p = 0.005$). Systemic toxicity was minimal in both groups.

Conclusions. LPSPs provide sensory blockade durations comparable to those from PLGA microspheres, with a smaller amount of drug loading. Motor blockade is shorter with LPSPs than with PLGA microspheres. LPSPs appear to be suitable for extended nerve blockade. Given their size and low density, they may be useful for topical anesthesia of the airway.

KEY WORDS: local anesthetics; sciatic nerve; controlled release.

INTRODUCTION

While local anesthetics are often intended to last for the relatively brief duration of a dental or surgical procedure (a few hours), there are many instances where more prolonged blockade is desirable. Controlled release technology can prolong drug effect and improve the therapeutic index, and therefore lends itself naturally to the problem of providing prolonged duration local anesthesia. A large number of such approaches have been tried (for examples, see refs. (1,3,9, 13)). In this article we describe the preparation of lipid-protein-sugar particles (LPSPs) made of dipalmitoylphosphatidylcholine (DPPC), albumin, and lactose, and their use as carriers for an amino-amide local anesthetic, bupivacaine.

Similar types of particles have been shown to produce effective drug release for approximately 24 hours *in vivo* when administered by inhalation (6). The duration of drug release from LPSPs is unlikely to rival that of particles based on more conventional sustained release materials such as high molecular weight α-hydroxy acids (e.g. poly(lactic-co-glycolic) acid, PLGA). However, total duration of release *in vitro* does not necessarily predict the duration of clinical effect. The duration of local anesthesia from a given particle type can be brief *in vivo* even though drug release might last weeks *in vitro* (3), suggesting that relatively high tissue levels are required for local anesthetic effect. The more rapid release of drug that can be expected from LPSPs may result in sustained high local levels of drug. Therefore, LPSPs could be suitable for prolonged local anesthesia, and might require a lesser drug loading to achieve an equivalent duration of clinical effect. They may also have advantages from a biocompatibility standpoint, since they are made from naturally occurring compounds and would be expected to have a shorter tissue dwell time than more conventional polymeric systems. There are two other features that make LPSPs potentially attractive. Similar particles (7) have been used for pulmonary delivery of local and systemic drugs, suggesting a potential use of these particles in airway diseases such as asthma, or to provide topical anesthesia for surgical procedures in the airway. Also, the method of production (spray drying) is amenable to scaling up.

In this report, we discuss the production and characterizion of LPSPs *in vitro,* then assess the *in vivo* local anesthetic efficacy of an optimal formulation in sciatic nerve block in the rat, using a neurobehavioural paradigm (15,19) that examines sensory (thermal nociception) and motor (weight bearing) function. The marked methodological variability between published reports on the *in vivo* effectiveness of controlled release local anesthetic preparations makes comparisons between them difficult. For this reason we compare the LPSPs to another delivery system in a series of experiments where the particles are delivered by the same means, to the same location, in equal quantity, and where the neurobehavioural outcomes are evaluated by the same methods and at the same time intervals in a blinded manner. We have selected large (20 to 120 μm) poly(lactic-co-glycolic) acid (PLGA)-based particles for this comparison because a) they have a long track-record of experimental use for anesthesia in the peripheral nervous system and spinal cord (2,3,5,8,16,17,20,21), and b) such microspheres have been described as producing very slow release of local anesthetics (3).

MATERIALS AND METHODS

Materials

Bupivacaine hydrochloride, human serum albumin (Fraction V), and lactose β-monohydrate were purchased from Sigma Chemical Co. (St. Louis, MO), L-α–dipalmitoylphosphatidylcholine (DPPC) from Avanti Polar Lipids (Alabaster, AL), poly (lactic-co-glycolic) acid (65:35, MW 110,000) (PLGA) from Medisorb, poly (vinyl alcohol) (88% hydrolyzed, MW 20,000) from Polysciences (Warrington,

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ABBREVIATIONS: LPSP, lipid-protein-sugar particle; PLGA, poly (lactic-co-glycolic) acid; DPPC, dipalmytoylphosphatidylcholine.

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PA), ethyl acetate and methylene chloride (both HPLC grade) from EM Sciences (Gibbstown, NJ), and USP grade ethanol from Pharmco Products (Brookfield, CT). Bupivacaine hydrochloride was made into the free base by alkaline precipitation and filtration. The ultraviolet absorbance spectrum from 200 nm to 300 nm, and a standard curve of absorbance at 272 nm vs. concentration were determined for each batch of the free base for quality control purposes.

Preparation of Spray-Dried Lipid-Protein Particles (LPSPS)

A 70:30 (v/v) ethanol:water solvent system was employed for solubilization and spray drying of excipients and bupivacaine. The solutions were prepared in the following manner: (i) the DPPC and bupivacaine free base were dissolved in a given amount of ethanol, (ii) the lactose and albumin were dissolved in a given amount of water (pH adjusted to 7.0), and (iii) the solutions were mixed immediately prior to spray drying. Solute concentrations ranged from 1 to 4 grams per liter. The proportion of albumin to lactose was kept constant in experiments where the DPPC content was changed.

Solutions were spray-dried using a Model 190 bench top spray drier (Büchi Co, Switzerland). The spray-drying parameters (inlet temperature, fluid flow rate, drying airflow rate, and aspirator pressure) were optimized based on the yield and size characteristics of both the blank (no bupivacaine) and the bupivacaine-containing particles. The optimized conditions were: inlet temperature = 115 to 120°C, solution feed rate = 12 to 14 ml/min, drying airflow rate = 600 l/min, and aspirator pressure = –18 barr. These conditions typically resulted in outlet temperatures in the range of 50 to 55°C for a given experimental run.

Particle Size and Bulk Density

A small amount of particles was dispersed in 20 ml of Isotoner (Coulter Corp., Miami, FL) and analyzed for size via a Coulter Multisizer (Coulter Electronics Ltd., Luton, U.K.) equipped with a 50 μm (for LPSPs) or 140 μm (for PLGA microspheres) orifice tube. Bulk mass density of the 60% DPPC powders was estimated using a Dual Platform Microprocessor Controlled Tap Density Tester (Vankel Technology Group, Cary, NC).

Scanning Electron Microscopy

The surface morphology of spray-dried particles was examined using a JEOL Model 6320 FV field emission scanning electron microscope (provided by the Massachusetts Institute of Technology Department of Materials Science and Engineering Electron Microscopy Center). Samples were mounted on stubs and coated with a layer of gold/palladium. Samples were scanned at voltages between 5 and 10 kV at a probe current setting of 3 and a working distance of 7 millimeters.

Bupivacaine Content of LPSPS

In order to determine the bupivacaine content of LPSPs, 10 mg of particles were agitated (Touch Mixer model 2332, Fisher Scientific, Pittsburgh, PA) for 20 seconds in 1 ml ethyl acetate. One-half ml of 0.1 N NaOH was then added, and the mixture was agitated for an additional 60 seconds. The suspension was centrifuged for 10 minutes at 14,000 rpm. One half ml of the upper organic layer was withdrawn, diluted with an equal volume of fresh ethyl acetate, and the absorbance at 272 nm was then measured (Cary 50 Bio UV-Visible Spectrophotometer, Varian, Australia) in a quartz cuvette (Hellma, Mullheim, Germany). Bupivacaine content was determined by comparison to a standard curve. Blank (no bupivacaine) LPSPs served as controls, and when processed in this manner had negligible absorbance at 272 nm. As an additional control we determined the amount of albumin that may have accompanied the bupivacaine in the ethyl acetate extraction (this was important because the two compounds have overlapping absorbance spectra), using a commercial kit (BCA Protein Assay Reagent Kit, Pierce Chemical Co., Rockford, IL). The amount of albumin was below the detection limit (< 25 μg/ml), and therefore could not account for measured absorbances at 272 nm.

In Vitro Release of Bupivacaine from Microparticles

Fifty mg of LPSPs or PLGA microspheres were suspended in 1 ml phosphate buffered saline pH 7.4 at 37°C and inserted into the lumen of a Spectra/Por 1.1 Biotech Dispodialyzer with an 8,000 MW cut-off. The dialysis bag was placed into a test tube with 12 ml phosphate buffered saline (PBS) and incubated at 37°C on a tilt-table (Ames Aliquot Mixer, Miles). At predetermined intervals, the dialysis bag was transferred to a test tube with fresh PBS. The bupivacaine concentration in the dialysate was quantitated by measuring absorbance at 272 nm and referring to a standard curve. Observation of the entire spectrum, and performance of a protein assay (as above) confirmed the absence of albumin from the samples that were measured.

Preparation and Characterization Of PLGA-Bupivacaine Microspheres

Microspheres loaded with 10% (w/w) and 50% (w/w) bupivacaine were prepared using a single emulsion method (3,22). Bupivacaine and PLGA were dissolved in methylene chloride, and the mixture was homogenized (Silverson L4R, Silverson Machines Ltd., Cheshire, England) in 50 ml 0.5% polyvinyl alcohol in 100 mM Trizma buffer pH 8.5 for 60 seconds. The resulting suspension was decanted into 100 ml of 0.05 % polyvinyl alcohol in 100 mM Trisma pH 8.5 and stirred for 3 minutes prior to rotary evaporation (Büchi Rotavap, Büchi, Switzerland) in a 37°C water bath until bubbling ceased. Spheres 20 μm to 120 μm in diameter were isolated by sieving (Newark Wire Co., Newark, NJ), then resuspended in 50 ml of water. The suspension was washed three times by centrifugation at 5000 rpm for 5 minutes. The final pellet was lyophilized to dryness.

Bupivacaine content was determined by dissolving 10 mg of microspheres in 1 ml methylene chloride, and comparing the resulting UV absorbance at 272 nm to a standard curve. Under similar conditions, PLGA microspheres containing no bupivacaine showed negligible absorbance at 272 nm.

Animal Care

Young adult male Sprague-Dawley rats weighing 310–420 g each were obtained from Taconic Farms (Germantown, NY), and housed in groups in a 6 AM–6 PM light-dark cycle.

Animals were cared for in compliance with protocols approved by the Animal Care and Use Committee at the Massachusetts Institute of Technology, and the Principles of Laboratory Animal Care published by the National Institutes of Health. Rats were only injected once.

Sciatic Blockade Technique

Prior to nerve block injections, rats were anesthetized briefly (< 2 minutes) with halothane. Concurrently, 75 mg of LPSPs or microspheres were suspended in 0.6 ml of carrier fluid (1% (w/v) sodium carboxymethyl cellulose, 0.1% (v/v) Tween 80) with gentle agitation (< 5 sec) in preparation for injection. A 20G needle was introduced postero-medial to the greater trochanter, pointing in an anteromedial direction (19). Once bone was contacted, the needle was withdrawn 1 mm and the particle-containing solution was injected. The left leg was always used for blocks; the right served as control.

Assessment of Nerve Blockade

The effectiveness of block was measured at various time points, applying the methods of Thalhammer et al. (19), or modifications thereof (15).

Nociceptive block was assessed by a modified hotplate test (18). Hind paws were exposed in sequence (left then right) to a hot plate at 56°C (Model 39D Hot Plate Analgesia Meter, IITC Inc., Woodland Hills, CA), and the time (latency) until paw withdrawal was measured by a stopwatch. Thermal latency is a measure of the intensity of analgesia. If the paw remained in contact for 12 seconds, it was removed by the experimenter to avoid injury to the animal or the development of hyperalgesia. This test was repeated three times for each rat at every time-point.

Motor strength was assessed by holding the rat with its posterior above a digital balance and allowing it to bear weight on one hindpaw at a time. The maximum weight that the rat could bear without its ankle touching the balance was quantified.

Neurobehavioural Data Processing

The data for nociceptive block are reported in terms of thermal latency (intensity) and duration of block. The duration of thermal nociceptive block is the time required for thermal latency to return to a value of 7 seconds (which is 50% of maximal block when a baseline thermal latency of approximately 2 seconds is taken into account). The duration of motor block was defined as the time for weight bearing to return halfway to normal from maximal block.

Statistical Analysis

Data are reported as means with standard deviations. Comparisons between groups were made using Student's t-test. These tests were unpaired except when comparing sensory vs. motor blockade in the same rat.

RESULTS

Production and Characterization of Lipid-Protein-Sugar Particles (LPSPs)

The spray-drying process conditions were initially optimized (with respect to yield) for the production of blank excipient particles (60:20:20 DPPC:albumin:lactose), as discussed in the methods section. (The reported percentage of DPPC refers to the composition of the excipients, excluding the delivered drug.) These conditions also appeared to be satisfactory for the production of the 10% (w/w) bupivacaine particles with varying DPPC contents. The results obtained from typical runs are shown in Table 1.

We measured the volume of fifty milligrams of each formulation loaded into test tubes, and calculated their densities. The 60% DPPC particles were less dense (0.07 ± 0.004 g/ml) than the 3% DPPC (0.24 ± 0.025, $p = 0.0007$) and 99% DPPC (0.14 ± 0.017, $p = 0.003$) particles. This difference in density was probably due to a difference in particle size (Table 1): the mean diameter of the 60% DPPC particles was greater than those of the 3% and 99% DPPC particles ($p = 0.00001$ and 0.000006 respectively). The 3% and 99% DPPC particles consistently formed macroscopic balls when stored as a dry powder, while this was not seen with the 60% DPPC particles. The impression of aggregation was confirmed by the observation that the average diameter of 3% and 99% DPPC particles rose to 19.28 ± 0.01 and 11.08 ± 0.34 μm respectively over a period of 4 weeks storage in a dessicator, while those of 60% DPPC particles did not change. The bupivacaine content of the various LPSPs formulations was similar (p = n.s.).

The 60% DPPC particles were spheroidal or, as can be seen in Fig. 1, concave in shape. Typical observed diameters were in the range of 3 to 5 microns. To ensure that the structural integrity of the particles was not impaired by the delivery method, we suspended 10 mg of particles in the carrier fluid (1% (w/v) carboxymethyl cellulose, 0.1% (v/v) Tween

Table 1. Characteristics of Lipid-Protein-Sugar-Particles (LPSPs) and PLGA-Based Microspheres

Microparticle	DPPC (%[a])	Bupivacaine loading (%)	n	Yield[b,c] (%)	Median diameter[b,d] (μm)	Bupivacaine content[b,e] (%)
LPSP	3	10	5	40 ± 6	2.58 ± 0.22	8 ± 0.4
	60	0	5	25 ± 5	4.66 ± 0.33	—
	60	10	5	37 ± 3	4.44 ± 0.39	8.8 ± 0.7
	99	10	5	37 ± 7	1.73 ± 0.05	7.6 ± 0.8
PLGA microsphere	0	50	6	53 ± 15	59 ± 12	44 ± 5

[a] Percentage of the total mass of excipient.
[b] Values indicated are means ± standard deviations.
[c] The fraction of solutes recovered, as weight % of the total amount of solutes taken in preparation.
[d] Volume-weighted.
[e] Measured as described in Methods.

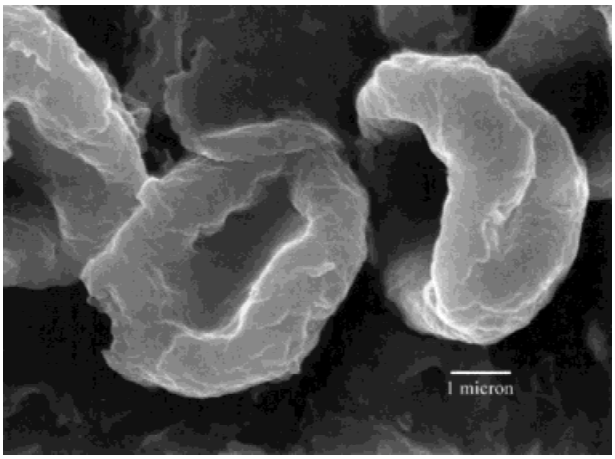

Fig. 1. Scanning electron micrograph of spray-dried lipid-protein particles (60% dipalmitoylphosphatidylcholine) as prepared.

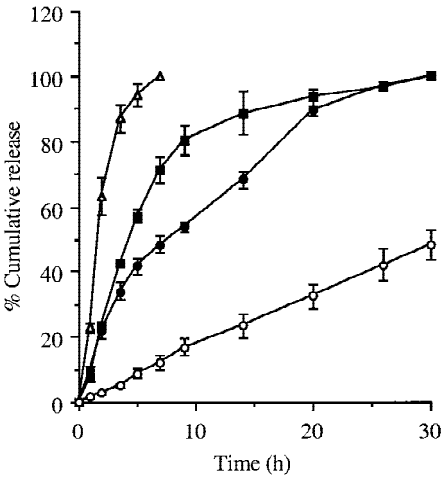

Fig. 2. Cumulative release from a dialysis tube of bupivacaine encapsulated in 10% (w/w) bupivacaine lipid-protein particles with 60% (●) or 99% (■) of the excipients being dipalmitoylphosphatidylcholine, or an equivalent amount of 0.5% (w/v) bupivacaine in solution (△). Also shown is release from 50% (w/w) bupivacaine PLGA microspheres (○). Data are means with standard deviations. n = 4 for all points.

80), mechanically agitated them for 10 seconds then injected them through a 20 G needle onto the inner wall of a test tube. Scanning electron microscopy demonstrated that the LPSPs' structure was not altered (not shown).

Production and Characterization of PLGA-Bupivacaine Microspheres

PLGA microspheres produced as described in the methods section appeared to be regular spheres as viewed by light microscopy (images not shown), with diameters more than ten times larger than those of the LPSPs. Data from microsphere production are in Table 1. The final particle yield was comparable to that of the spray-dried particles. The data relevant to the production of the10% (w/w) bupivacaine microspheres were similar to those for the 50% (w/w) microspheres, and their mean bupivacaine content (w/w) was 8% (n = 2).

Bupivacaine Release from LPSPs

These experiments were performed so as to allow rational selection of a particular formulation for use in *in vivo* studies.

Pilot studies had shown that 3% DPPC particles almost completely disappeared by 18 hours after suspension in phosphate buffered saline, while 60% and 99% particles lasted many days. Consequently, we focused on the latter preparations. Bupivacaine release from 50 mg samples of 10% loaded (w/w) bupivacaine-LPSPs (n = 4 for each particle formulation) was measured. Figure 2 shows the cumulative release of bupivacaine over time. Both particle types caused delayed release of bupivacaine into the dialysate compared to the unencapsulated drug (1 ml of 0.5% (w/v) bupivacaine, or 5 mg). Both 60% and 99% DPPC particles completely released their bupivacaine content within 24 hours. However, release from the 60% DPPC particles was more gradual: at 9 hours, the 60% DPPC particles had released 53.8 ± 1.5% of their bupivacaine content, whereas the 99% DPPC particles had released 80.6 ± 4.7% ($p = 0.0002$). Consequently, the 60% DPPC formulation was selected for *in vivo* studies. Figure 2 also shows the release of bupivacaine from 50% (w/w) PLGA particles (n = 4). The release, on a percentage basis, was much slower than that from LPSPs: less than 50% of total drug content was released in 30 hours, at which time the LPSPs had released 100% of drug content. The total amount of drug released by the LPSPs was slightly greater than that released by PLGA microspheres at most early time points (by 3.5 hours, the LPSPs had released 1.65 ± 0.17 mg of bupivacaine vs. 1.26 ± 0.15 mg for PLGA microspheres, $p = 0.01$). This relationship was reversed at longer durations (by 9 hours the LPSPs had released 2.6 ± 0.2 mg of bupivacaine, compared to 4.2 ± 0.7 mg for the PLGA microspheres ($p = 0.02$)).

Effectiveness of Sciatic Nerve Block

Rats were injected at the sciatic nerve with 75 mg (~ 215 mg/kg) of spray-dried LPSPs containing 10% (w/w) bupivacaine, and the time course of nerve blockade was followed. All rats injected with 10% (w/w) bupivacaine LPSPs achieved maximal nerve block (thermal latency = 12 seconds) by the time of the first testing (30 minutes). Four out of ten rats injected with 50% (w/w) bupivacaine PLGA microspheres did not achieve maximal block by that time. Nine out of ten rats injected with 50% (w/w) bupivacaine PLGA microspheres had maximal block by one hour after injection. All achieved maximal block within 3 hours.

The average duration of thermal nociceptive block from 10% (w/w) bupivacaine LPSPs was 468 ± 210 min (n = 10). The duration of thermal nociceptive block obtained from injection with 75 mg of PLGA microspheres with 50% (w/w) loading of bupivacaine was 706 ± 344 min (n = 10). This was not statistically different from the duration obtained with the 10% (w/w) bupivacaine LPSPs ($p = 0.08$).

In order to compare the efficacy of equal loading with bupivacaine, rats were injected with 75 mg of 10% (w/w) bupivacaine PLGA microspheres (n = 5), and 50% (w/w) bupivacaine LPSPs (n = 2). The former did not result in nerve block as defined by our paradigm, while the latter caused rapid demise of the rats.

We injected 6 rats with 75 mg of blank DPPC-albumin-

lactose particles in order to verify that the increased efficiency (comparable duration of block with much lower drug loading) of the LPSPs over bupivacaine microspheres was not due to an intrinsic nerve blocking-effect of the component excipients. Blank LPSPs did not produce any detectable nerve block.

Modality-Specificity of Nerve Blockade

Blank LPSPs and 10% (w/w) bupivacaine PLGA microspheres did not cause any impairment in sensory or motor function. Motor blockade from 10% (w/w) bupivacaine LPSPs lasted 508 ± 258 min, while that from 50% (w/w) bupivacaine microspheres lasted 1062 ± 456 min ($p = 0.005$). Fig. 3 focuses on the clinically important comparison of the durations of motor block (x-axis) and sensory block (y-axis) for both preparations. The motor block from the PLGA microsphere preparation lasted 50% longer than did the sensory block ($p = 0.003$), as evidenced by the location of the representative point below the line of unity. The LPSPs had durations of sensory and motor block that were not statistically different from each other (8% difference, $p = 0.37$).

Systemic Distribution of Bupivacaine

We used the presence of functional deficits in the uninjected extremity as a measure of the degree of systemically distributed local anesthetic (i.e. toxicity). Thermal latency (the length of time that a rat would leave his paw on the hotplate) was measured in the un-injected leg at predetermined intervals, in rats who received 10% (w/w) bupivacaine LPSPs or 50% (w/w) bupivacaine PLGA microspheres (Fig. 4). There was no statistically significant difference between the mean latencies in the two groups at any time point.

One rat (out of 11) injected with 50% (w/w) bupivacaine PLGA microspheres died, approximately 2 hours after injection. Necropsy revealed congestion of the liver, and kidneys, most consistent with heart failure. Both rats injected with 50% (w/w) bupivacaine LPSPs died. There were no deaths in the 10% (w/w) bupivacaine LPSP group ($n = 10$), or 10% (w/w) PLGA microsphere group.

Encapsulation improved the safety and efficacy of bupivacaine. None of the rats injected with 10% (w/w) bupivacaine

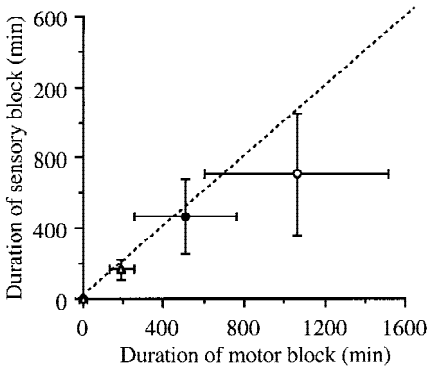

Fig. 3. Comparison of the durations of sensory and motor blockade for 10% (w/w) bupivacaine lipid-protein particles (●), 50% (w/w) bupivacaine PLGA microspheres (○), or 0.5% (w/v) bupivacaine in solution (△). Points falling above the diagonal line bisecting the graph represent a relative sensory predominance in nerve blockade, while those falling below have motor predominance.

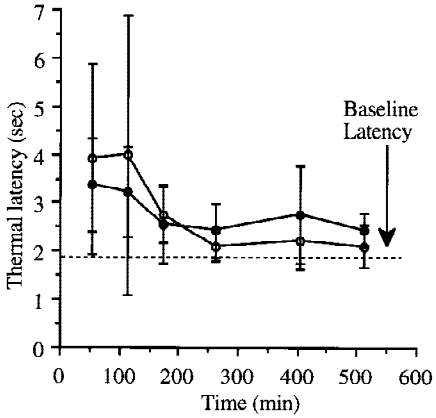

Fig. 4. Time course of thermal latency in the uninjected leg following sciatic nerve block, in animals injected with 10% (w/w) bupivacaine lipid-protein particles (●) or 50% (w/w) bupivacaine PLGA microspheres (○). Here thermal latency in the uninjected (contralateral) leg is used as a measure of systemic drug distribution. Data are means with standard deviations. None of the differences in latency between the two groups were statistically significant.

LPSPs had marked increases in contralateral latency. In comparison, rats ($n = 6$) injected with an equivalent amount of bupivacaine in solution (1.5 ml of 0.5% bupivacaine, i.e. 7.5 mg) had a duration of block of 166 ± 55 min. For this experiment we used larger rats (approx. 410 g) than those used in the remainder of the study, in order to avoid animal death (the median lethal dose of bupivacaine in adult rats is 30 ± 5 mg/kg (14), or 10.5 mg in a 350 g rat). Even so, one of those rats had severe signs of systemic toxicity (thermal latency = 12 seconds in the uninjected leg). It was not possible to directly compare the efficacy of bupivacaine solution and 50% bupivacaine microspheres, since the dose of bupivacaine contained in 75 mg of those microspheres (38.5 mg) is approximately three times the median lethal dose of the unencapsulated drug (14). Nevertheless, it is obvious that the microspheres increased the safety of bupivacaine.

DISCUSSION

Of the three LPSP formulations tested *in vitro,* the 60% DPPC particles appeared optimal in terms of drug release. The slower release of bupivacaine from the 60% DPPC particles compared to the 99% DPPC particles was somewhat surprising; *a priori* one might have expected the more hydrophobic nature of the latter particle type to delay release to a greater extent. This discrepancy may be related to the larger size of the 60% DPPC particles, which may impede access of water to the encapsulated drug and of drug to the exterior. Bupivacaine binding by albumin may also play a role.

The DPPC-albumin-lactose particles appear to be effective as vehicles for the local delivery of percutaneously injected local anesthetics in rats. The LPSPs had a more rapid onset of nerve block than the PLGA microspheres, which may be a reflection of the initial more rapid release of drug from the LPSPs. They were as effective as PLGA microspheres in terms of duration of local anesthesia, with one-fifth the initial loading of drug. (The duration of block that we obtained with the 50% bupivacaine microspheres is considerably longer than previously published values. Seventy-five percent loaded particles have been reported to last 6.0 ± 3.0

hours (3), compared to 11.8 ± 5.7 hours for the 50% loaded particles in this study.) It would appear, based on the *in vitro* release studies, that this improved ratio of duration of block to drug loading most likely stems from the proportionally more rapid release of drug from the LPSPs. An alternative explanation would be that the LPSPs themselves have an effect on nerve function. While we cannot exclude this possibility, LPSPs without bupivacaine did not to cause any detectable deficits in nerve function.

The more rapid fractional release of drug from the LPSPs did not result in greater toxicity than occurred from PLGA microspheres, as evidenced by the fact that thermal latency in the uninjected leg was not increased. (Increases in contralateral latency are an early sign of severe local anesthetic toxicity (14).) The *in vitro* data suggest that this was because the discrepancy in total drug release between the two particle types was not as great as the fractional (percentage) difference. PLGA microspheres would appear to provide a better margin of safety at high bupivacaine loadings.

In general, it is not desirable for motor block to be of longer duration than sensory block (resulting in a paralyzed limb with full sensation). In fact, there are applications (such as obstetric anesthesia) where sensory block in the absence of motor block is desirable (so the mother can push during labor while still obtaining pain relief). The LPSPs had a more favorable ratio of duration of sensory to motor block than did the PLGA microspheres. The explanation for this difference is most likely to be pharmacokinetic. The large myelinated fibers ("A fibers") that mediate motor function are more sensitive to amino-amide local anesthetics than are the small unmyelinated fibers that mediate pain ("C fibers") (23). Thus, one would expect sensation to return before motor function. In the case of the PLGA microspheres, the rate of decline of the local concentration of bupivacaine is probably slower, so that the time interval between the termination of sensory blockade and motor blockade is longer. The kinetic argument for the difference between the functional selectivities of LPSPs and PLGA microspheres is supported by the observation that bupivacaine solution (in the absence of any controlled release device) also shows approximately equal durations of sensory and motor block (Fig. 3), as has been previously noted in this animal model (14,15).

It bears mentioning that the LPSPs are of a size and density that makes them suitable for inhalational delivery. (The tap density - a more standardized measure of particle density - of the 60% DPPC LPSPs was 0.11 ± 0.04 g/ml.) Nebulized local anesthetics have been used in a variety of roles in the management of medical problems of the upper airway and pulmonary tree (12), including the management of asthma (4,11). Nebulized lidocaine results in lower serum levels of drug than are achieved by equieffective intravenous doses (10). Thus it is conceivable that particles of this sort could be beneficial in severe asthma, or in blunting patient responses to intratracheal suctioning, bronchoscopy, and other noxious procedures.

In summary, controlled release of bupivacaine using lipid-protein-sugar particles can provide prolonged duration local anesthesia that is as effective (depth and duration of anesthesia) as that conferred by conventional polymer-based particles. The potential for improved long-term biocompatibility merits investigation; if confirmed, this would suggest that LPSPs could present advantages in providing prolonged duration anesthesia in the clinical setting.

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