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Prolonged Regional Nerve Blockade

Injectable Biodegradable Bupivacaine/Polyester Microspheres

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Background: **Biodegradable microspheres are a useful method of drug delivery because they are both injectable and biodegradable, eliminating the need for surgical implantation or removal. Previous work has characterized implantable preparations of local anesthetics in polymer pellets for prolonged regional anesthesia. In this article, the authors characterize injectable suspensions of bupivacaine-polymer microspheres and examine whether they can produce prolonged blockade of the sciatic nerve in rats.**

Methods: **Microspheres were prepared using polylactic-co-glycolic acid polymers loaded with 75% w/w bupivacaine by a solvent evaporation method. Bupivacaine release from microspheres was determined *in vitro* by ultraviolet spectroscopy and scintillation counting. Sensory and motor blockade of the rat sciatic nerve were assessed *in vivo* after injection of microsphere suspensions.**

Results: **Depending on the type of microspheres, the dose, and the additive used, mean duration of sciatic nerve block ranged from 10 h to 5.5 days. Incorporation of 0.05% w/w dexamethasone into the microspheres resulted in significant prolongation of block (up to 13-fold), and only preparations that contained dexamethasone produced blocks lasting beyond 1 day. Bupivacaine was released in a controlled manner *in vitro*. Dexamethasone does not substantially slow bupivacaine release from microspheres *in vitro*.**

Conclusions: **Prolonged percutaneous blockade of peripheral nerves is feasible. The recovery from blockade is complete, and plasma bupivacaine levels are far below the range associated with systemic toxicity. The mechanisms underlying the dexamethasone block-prolonging effect are under investigation. (Key words: Anesthesia: regional. Anesthetics, local: bupivacaine; dexamethasone. Drug delivery: sustained-release. Microspheres: biodegradable polyester. Nerves, sciatic: blockade.)**

PROLONGED regional blockade may have useful applications for both acute and chronic pain management. Currently available local anesthetics rarely last beyond 12 h, unless catheter infusions are used.[1] Previous work in our laboratories has shown the feasibility of prolonged regional blockade using surgically implantable pellets consisting of polymer-local anesthetics matrixes. These comprised either dibucaine or bupivacaine in polyanhydride polymer pellets[2,3] or either bupivacaine or tetracaine in polylactic-co-glycolic acid polymer microspheres.[4] In these studies, sensory and motor blockade lasted for periods of 1–10 days, depending on the type of preparation and dose used.

Although there may be clinical indications for surgical implantation of sustained-action local anesthetics, the breadth of application of these agents, particularly

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by anesthesiologists, would be greatly enhanced by development of injectable preparations.

Biodegradable microspheres have been shown to be an attractive alternative to implants. They are generally formulated by a solvent evaporation method[5] and have been used to release drugs for potential treatment of a range of illnesses.[6–9]

Previous clinical uses of microspheres has applied mainly to high potency drugs, which require release of micrograms per day, and therefore have drug/polymer weight ratios less than 10–12%.[6–9] Local anesthetics are comparatively low potency drugs, and require milligrams to tens of milligrams per hour to maintain blockade of nerves. Thus, to make clinically useful microspheres with local anesthetics, it becomes necessary to develop microsphere formulations with previously unattainably high drug loadings, that is, 50–75% drug to polymer weight to weight ratio (w/w).

The homopolymer polylactic acid is abbreviated PLA. Polylactic-co-glycolic acid (PLGA) is a random copolymer polymerized from lactic and glycolic acids. The ratios 75/25, 65/35, and 50/50 refer to the molar ratios of lactic to glycolic acid repeating units. Polylactic and lactic-glycolic acid polymers are widely used in drug delivery as well as in a variety of implantable devices and sutures.[8,9] There is extensive evidence attesting to their safety, their biocompatibility, and their generation of a very mild tissue reaction.[10] Previous work by Le Corre *et al.*,[11] and Malinovsky *et al.*[12] examined spinal and epidural administration of local anesthetic microspheres, but with much lower drug loadings resulting in a much shorter duration of effect (less than 6 h) than that seen in the current work.

In the current study, we test the following hypotheses:

1. Bupivacaine-polyester microspheres can be formulated with mechanical stability at a very high percentage of drug loading, *i.e.,* up to 75% by weight.
2. Bupivacaine-polyester microspheres with a high percentage of loading have controlled release of drug, and do not produce rapid initial burst release of drug *in vitro* or *in vivo*.
3. Bupivacaine-polyester microspheres can produce sciatic nerve blockade with a wide margin of safety regarding systemic toxicity.
4. The duration of sciatic blockade from bupivacaine-polyester microspheres is prolonged by incorporation of dexamethasone into the microspheres.

Materials and Methods

Materials

The microspheres containing nonradiolabeled bupivacaine and nonradiolabeled dexamethasone used in this study were supplied by Medisorb (Cincinnati, OH) and were all formulated using a solvent evaporation method. A list of the microspheres used, their batch number, and contents are shown in table 1. The 65/35 PLGA (lot. no. S 2170 Si 177, Mw 130,000) was supplied by Medisorb. Polyvinylalcohol (Mw 30,000–70,000, Lot No. 93H0967) was supplied by Sigma (St. Louis, MO). Tritium-labeled dexamethasone was obtained from Amersham (Arlington Heights, IL) (specific activity 9.24×10^{10} dpm/μM) and tritium-labeled bupivacaine was donated by Dr. Gary Strichartz from Brigham and Womens Hospital, Boston (specific activity 1.71×10^{11} dpm/μM). Bupivacaine free base was produced and supplied by Purdue Pharma (Yonkers, NY; lot no. 32931), and dexamethasone was supplied by Sigma (lot no. 34H0502). Trisma base was supplied by Sigma (lot no. 64H5732). Dulbecco's phosphate buffered saline was supplied by Gibco (Gaithersburg, MD; lot no. 14N5447), (KCL 2.68 mM/l, KH_2PO_4 1.47 mM/l, NaCl 547.5 mM/l, $NaHPO_4$ 9.50 mM/l). The suspension/injection medium used in the *in vivo* experiments was supplied by Medisorb and consisted of 0.5% w/v sodium carboxymethylcellulose (low viscosity) and 0.1% w/v Tween 80 and 0.18% w/v methyl paraben.

Polymer Synthesis

Polylactic acid and polylactic-co-glycolic acid were synthesized by ring opening polymerization of their cyclic dimers.[13,14] The PLA used in this study were racemic mixtures of dextro (D) and levo (L) rotamers. Racemic PLA is less crystalline than the stereoregular D-PLA and L-PLA. In addition, copolymers of latic-co-glycolic acid are less crystalline than the PLA homopolymer. The rate of polymer degradation is inversly proportional to the degree of crystallinity, and proportional to the amount of glycolide present in the polymer. All the polymers had a molecular weight greater than 100,000 (determined by gel permeation chromatography) to facilitate the encapsulation of the high percentage drug loadings used in this study.

Microsphere Formulation and Local Anesthetic Incorporation

Unlabeled Microsphere Preparation. Microspheres containing bupivacaine and bupivacaine plus

Table 1. The Microsphere Formulations Tested *In Vitro* and *In Vivo*

Medisorb Batch No.	Polymer (DL)	Mass Median Diameter (μm)	% Dexamethasone (weight/weight)	% Bupivacaine (weight/weight)
177	65/35	73	0	75
181	65/35	76	0.05	74
190	65/35	ND	0	0
197	65/35	ND	0.05	0
167	75/25	76	0	74
183	75/25	75	0.05	73
188	75/25	ND	0	0
194	75/25	ND	0.05	0
173	100	75	0	70
179	100	75	0.05	68
186	100	ND	0	0
192	100	ND	0.05	0
249	50/50	82	0	72
253	50/50	40	0.05	70
256	65/35	48	0.05	73
242	65/35	80	0.05	73
258	65/35	48	0.005	72

ND = not determined.

dexamethasone were prepared by a solvent evaporation process. An oil-in-water emulsion was formed as follows: 15 g Medisorb 65/35 PLGA and 45 g bupivacaine base were dissolved in 370 g ethyl acetate. Dexamethasone was added to the organic phase when included in the microspheres. The emulsion was formed by adding Tris buffered (*p*H 8.0–8.5) 1% polyvinylalcohol, and then hardened in Tris buffered water (*p*H 8.0–8.5) for 1 h. The solid product was filtered through sieve screens, and the fraction between 25 μm and 125 μm was collected and air dried. Bupivacaine content was determined by high-performance liquid chromatography, using acetonitrile as the mobile phase and an ultraviolet detector at 263 nm.§§

Labeled Microsphere Preparation. The radiolabeled microspheres were formulated by a single emulsion technique, using an evaporation process.[9,15] Two types of radiolabeled microspheres were formulated, one that contained 75% w/w unlabeled bupivacaine and 0.05% w/w tritium-labeled dexamethasone and the other contained 0.05% w/w unlabeled dexamethasone and 75% w/w tritium-labeled bupivacaine. The microspheres that contained tritium-labeled dexamethasone were prepared as follows: an aliquot of dexamethasone containing 8×10^6 disintegrations per min (dpm) was added to 100 μl of a solution of 5 mg unlabeled dexamethasone in 5 ml ethanol. The sample was dried under a stream of nitrogen for 1 h, and 50 mg 65/35 PLGA and 150 mg bupivacaine free base in 1 ml methylene chloride was added. The tube was vortexed for 1 min at 200 rpm on a Fisher Scientific Touch Mixer (Pittsburgh, PA; model 232). Then 1 ml 0.3% polyvinylalcohol in 100 mM Trisma (tris(hydroxymethyl)amino methane) base (*p*H adjusted to 8.4) was added, and an emulsion formed by vortexing for 45 s. The emulsion was then poured into 100 ml 0.1% polyvinylalcohol in 100 mM Trisma base. The methylene chloride was removed from the microspheres using a rotary evaporator under vacuum at 40°C for 20 min. The microspheres were strained through a series of stainless steel sieves of pore sizes 140 μm, 60 μm, and 20 μm (Newark Wire, Newark, NJ). Those microspheres that were smaller than 20 μm and larger than 140 μm in diameter were discarded. The microspheres that fell in the size range 20–140 μm were centrifuged at 4,000 rpm for 10 min, rinsed with buffer, and centrifuged three times. The microspheres were then frozen in liquid nitrogen and lyophilized overnight.

The microspheres that contained tritium-labeled bupivacaine were formulated as described earlier with the following exceptions: an aliquot of bupivacaine in ethanol consisting of 9×10^6 dpm was added to 150 mg bupivacaine free base. The solution was then vortexed

§§ United States Pharmacopoeia Method for Bupivacaine hydrochloride injections 227.

to ensure homogeneous mixing of labeled and unlabeled bupivacaine. The ethanol was removed under a stream of nitrogen for 1 h. On removal of ethanol, 50 mg 65/35 PLGA and 100 μl dexamethasone solution (5 mg dexamethasone in 5 ml ethanol) were added. Thereafter, the protocol was the same as that used to formulate microspheres that contained radiolabeled dexamethasone.

To determine drug content, 5 mg microspheres were dissolved in 2 ml methylene chloride and the local anesthetic concentration determined by ultraviolet spectroscopy. The absorbance at 272 nm was read and compared to a calibration curve of known amounts (0–2.5 mg/ml) of bupivacaine free base dissolved in 2 ml methylene chloride. Release media, incubated with control microspheres containing polymer but no bupivacaine, showed insignificant absorbance at 272 nm.

Morphologic Evaluation

Microspheres were examined morphologically at various stages of preparation, purification, and storage. Light microscopy (American Optical One-Ten) at 10–20× magnification was used to screen formulations to ensure that crystalline bupivacaine did not leach during formulation. An Electroscan (Wilmington, MA) environmental scanning electron microscope was used to examine completed formulations for shape, porosity, and size. Microspheres were placed on an adhesive medium and examined at magnifications ranging from 200× to 1500×.

A Coulter Multisizer II (Coulter Electronics, Luton, England) was used to determine the mass median diameter of the microspheres by light scattering. These measurements were corroborated using a HIAC-Royco particle counter 9060 (Pacific Scientific).

In Vitro *Release Studies*

Unlabeled Microspheres. Microspheres (5 mg per tube) were incubated with 2 ml Dulbecco's phosphate-buffered saline at 37°C, *p*H 7.4, with 0.1% w/v sodium azide added as an antifungal agent. Centrifugation (14,000 rpm for 5 min) was used to separate microspheres from buffer-containing released bupivacaine. The buffer was changed at 0.5, 2, 6, 12, 24 h and once daily thereafter. The bupivacaine concentration in the buffer was determined using absorbance at 272 nm *via* a Hewlett Packard 8452 Diode Array Spectrophotometer. Four replicates from each batch of microspheres were assayed.

Labeled Microspheres. The procedure used to determine the *in vitro* release of both bupivacaine and dexamethasone was the same as that used for nonradiolabeled microspheres, except that the amount of radiolabeled compound released into the buffer was determined by scintillation counting using a LKB Wallac 1214 Rackbeta Liquid Scintillation Counter after addition of Ecolume scintillation fluid. The counting efficiency (counts per min/disintegration per min) was determined to be 51%. Five replications of each set of radiolabeled microspheres were used.

Preparation and Injection of Microsphere Suspensions for Sciatic Nerve Blockade In Vivo

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing between 200 and 350g were used. Rats were handled daily and habituated to the testing paradigm (see Testing for Sciatic Nerve Block, later) before exposure to local anesthetic injections. Rats were anesthetized with halothane 2–3% inspired concentration in oxygen *via* face mask during injections.

The dose used ranged from 50 to 600 mg bupivacaine/kilogram of rat. The injection volume used was 0.6 ml in all cases. The microspheres in the suspending media were vortexed at maximum speed for 2 min before injection. Percutaneous sciatic nerve blockade was performed by a posterior approach immediately caudad to the sciatic notch, with skin entry medial and cephalad to the greater trochanter. Technical failure (defined as no measurable block of sensory or motor function after injection) occurred in 5% of injections, and these animals were eliminated from the study.

Testing for Sciatic Nerve Block

Sensory and motor blockade were measured as described previously.[1,16] Blockade of thermal nociception was determined with a Life Science Model 35D hotplate at 56°C using 12 s as a cutoff latency to prevent thermal injury or hyperalgesia.[17] The hot plate was equipped with a light emitting diode accurate to ± 0.1°C. In addition, the accuracy of the light emitting diode was checked using a thermocouple.

The very high testing temperature of 56°C was used because it represents a strong stimulus and most clearly distinguishes full sensory blockade from milder analgesic effects. Intact unmedicated rats withdraw their paw from the plate within 1–3 s. We defined sensory block duration as the mean duration of time for which the latency of a group of five rats was greater than or equal to 7 s. Seven seconds is midway between the cutoff latency of 12 s and a typical (unanesthetized) baseline latency of 1–3 s.

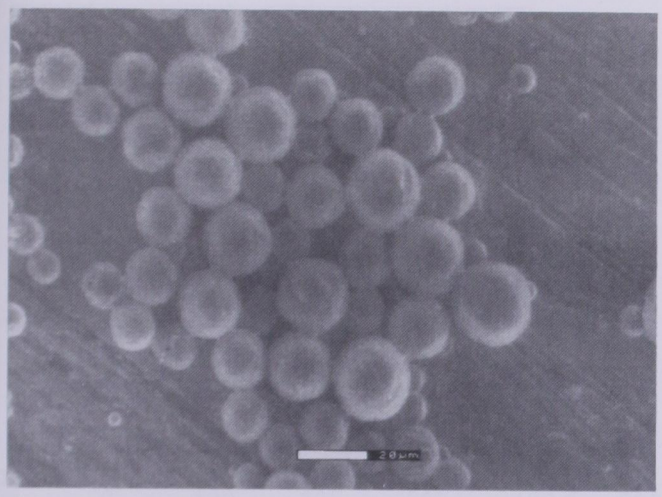

Fig. 1. Environmental scanning transmission electron micrograph of polylactic-co-glycolic acid microspheres loaded with 75% w/w bupivacaine and 0.05% w/w dexamethasone.

Note that this test is quite different from the common version of a hot-plate test,[18] which involves placing a rat or mouse with all four extremities on the hot plate. In our test, only a single hind leg is placed on the hot plate at each measurement, with the contralateral leg placed on a wooden block at room temperature. By alternating sides, the contralateral leg serves as a within-subject control to detect potential systemic analgesic effects or stress-induced analgesia.

In addition to thermal sensory testing, motor testing was performed at each time point to examine the rats' ability to hop and to place weight on its hind leg.[1,16,17] Duration of motor blockade is defined as the mean time for return to a motor score of 2 on the 1–4 scale. Institutional approval was requested from The Animal Care and Use Committee, at the Children's Hospital, Boston and was subsequently granted. Animals were handled and cared for according to institutional, state, and federal regulations, and according to the guidelines of the International Association for the Study of Pain.

As a control for blocking effects of microspheres in the absence of bupivacaine, placebo microspheres of each polymer type and microspheres loaded with 0.05% dexamethasone but no bupivacaine were formulated and tested (n = 4 for each preparation).

Plasma Bupivacaine Concentrations

Blood samples were obtained both by tail vein and by cardiac puncture at the time of death. Samples were placed into tubes anticoagulated with ethylene diamine tetraacetic acid, centrifuged to separate plasma, and stored frozen until analyzed. The bupivacaine content was determined using high-performance liquid chromatography equipped with a reverse phase column, the detection limit was 0.005 μg/ml.

Statistical Methods

For *in vitro* release experiments with various microsphere preparations, the percent cumulative release at 6 days of incubations was compared between different formulations using analysis of variance, with Scheffe's tests for *post hoc* groupwise comparisons.

Comparisons among groups of rats receiving different formulations were made using analysis of variance or repeated-measures analysis of variance, with Scheffe's tests or Bonferroni corrected paired or unpaired *t* tests for *post hoc* groupwise comparisons.

Results

Preparative Analyses

A list of the unlabeled formulations tested, their bupivacaine content, and the mass median diameter is given in table 1. The product yield of these microspheres in the 25–125 μm range averaged 78%. The yield of microspheres that contained unlabeled bupivacaine and radiolabeled dexamethasone (weight of bupivacaine + weight of polymer/weight of microspheres) averaged 45%, and the bupivacaine content was 75 ± 2%. The yield of microspheres containing radiolabeled bupivacaine and unlabeled dexamethasone was 50%, and the bupivacaine content was 72 ± 2%.

Microsphere Morphology

Figure 1 shows an environmental scanning electron micrograph of 65/35 PLGA loaded with 75% w/w bupivacaine and 0.05% w/w dexamethasone. All the formulations made with the methods outlined in Materials and Methods showed smooth round microspheres without evident pores. There was no evidence of crystalline bupivacaine by microscopy for any of the formulations shown. It should be noted that certain alternative formulation methods not described here resulted in a high incidence of leaching with bupivacaine crystal formation.

In Vitro *Release Kinetics*

The *in vitro* release of bupivacaine from microspheres made with the polymers 50/50 PLGA, 65/35

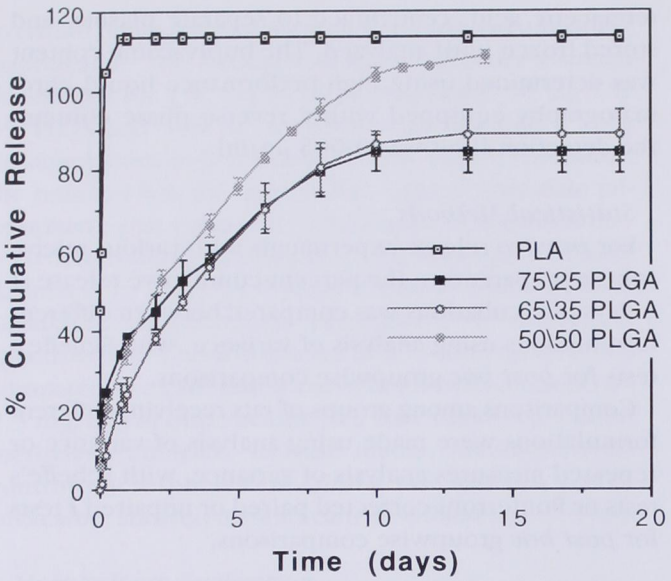

Fig. 2. The percent cumulative release *versus* time in the *in vitro* release study of PLA, 75/25 polylactic-co-glycolic acid, 65/35 polylactic-co-glycolic acid, and 50/50 polylactic-co-glycolic acid microspheres loaded with 75% bupivacaine. Error bars indicate standard errors; n = 4.

PLGA, 75/25 PLGA, and PLA is shown in figure 2. Values shown represent data uncorrected for background interference with the absorption measurements, so that

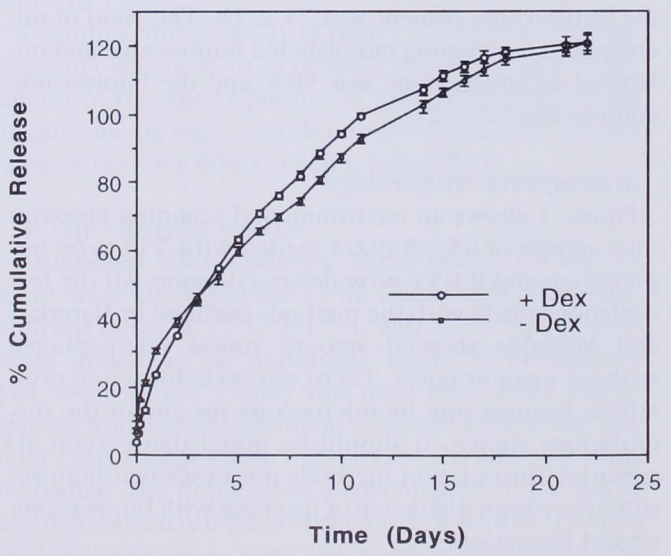

Fig. 3. A comparison of the percent cumulative release *versus* time in the *in vitro* release study of two formulations of polycactic-co-glycolic acid, one of which had 0.05% w/w dexamethasone incorporated in the microspheres and one that did not. Error bars indicate standard errors; n = 4.

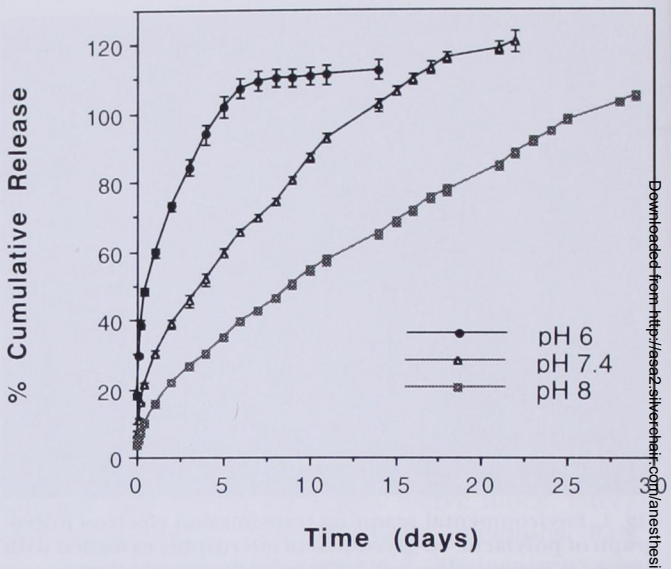

Fig. 4. The percent cumulative release *versus* time in the *in vitro* release study of polylactic-co-glycolic acid microspheres loaded with 75% bupivacaine at *p*H levels 6, 7.4, and 8. Error bars indicate standard erros, n = 4.

apparent greater than 100% cumulative release represents accumulated errors of up to 20 time points. Bupivacaine release was fastest from PLA microspheres ($P < 0.05$ for PLA in pairwise comparison to each of the other polymers; analysis of variance-Bonferroni) and similar for the other three polymers (P = NS for each paired comparison). Figure 3 shows the rates of *in vitro* release of bupivacaine from 75/25 PLGA microspheres loaded with 75% bupivacaine with or without dexamethasone. Bupivacaine is released at similar rates in both cases, so the prolonging effect of dexamethasone on block duration *in vivo* (see later) is not adequately explained by the *in vitro* results. No burst release was observed in any formulation tested, indicating that the microspheres were very stable. Figure 4 compares the percent cumulative release of bupivacaine from microspheres when the *p*H levels of the buffered medium were 6, 7.4, and 8, respectively. The release rate of bupivacaine was greater at *p*H 6 than at *p*H 7.4 or 8 ($P < 0.001$ for each paired comparison). Bupivacaine has a greater ionized fraction and greater aqueous solubility at *p*H 6 than at *p*H 7.4 or 8.

Radiolabeled Microspheres

The *in vitro* release curves obtained from microspheres containing unlabeled bupivacaine and radiolabeled dexamethasone proved that dexamethasone was incorporated into the microspheres. Microspheres that

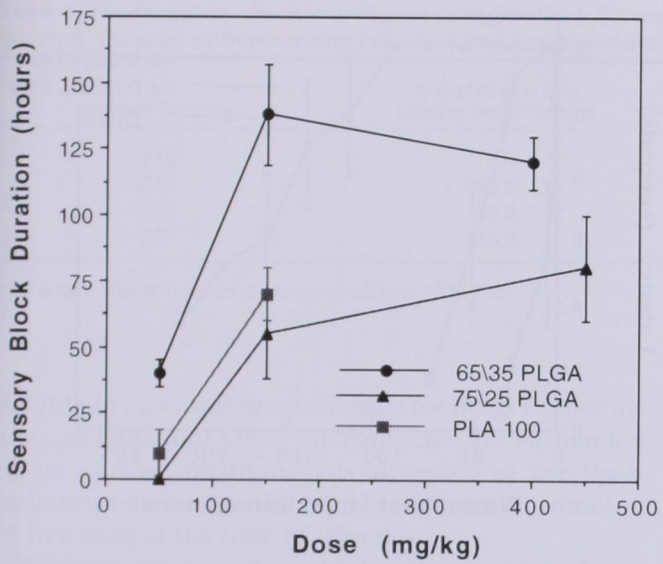

Fig. 5. Dose-response curve (duration of sensory blockade *vs.* dose) for polylactic acid, polylactic-co-glycolic acid 65/35 and 75/25 polylactic-co-glycolic acid microspheres loaded with bupivacaine 75% w/w and dexamethasone 0.05% w/w, administered at doses of 50 to 450 mg of bupivacaine/Kg of rat. Error bars indicate standard errors, n = 4 or 5 in each case.

had one of the two drugs radiolabeled and the rate of release of radiolabeled dexamethasone was compared to that of radiolabeled bupivacaine. Both drugs were released at similar rates, and its rate of release was comparable to that of bupivacaine. Release of bupivacaine assayed by ultraviolet spectroscopy and by scintillation counting was identical within experimental error (data not shown).

Rat Sciatic Nerve Blockade In Vivo

A plot of duration of sensory block *versus* dose for three polymer formulations is shown in figure 5. Based on this dose-response, a dose of bupivacaine 150 mg/kg was chosen for subsequent experiments. This dose is far below the threshold for systemic toxicity (see later).

Groups of rats received formulations of 150 mg/kg bupivacaine in PLA, 75/25 PLGA, 65/35 PLGA, or 50/50 PLGA with or without 0.05% w/w dexamethasone. In each case, the presence of dexamethasone in the microspheres resulted in a 6–13-fold increase in the duration of block, as shown in table 2.

‖‖ Feldman HS: Doctoral Thesis. The relative acute systemic toxicity of selected local anesthetic agents. Department of Anesthesia and Intensive Care, Uppsala University, Sweden, 1989.

Groups of rats (total n = 30) received sciatic blockade with 600 mg/kg bupivacaine in each of the above polymer formulations. Many showed evidence of impending or minor systemic toxicity, with 1–4 h of somnolence, but in no case was there loss of righting reflex, death, visible convulsions, or evidence of systemic analgesia by contralateral leg testing on the hot plate. This dose is at least 30–150 times the convulsant dose of aqueous bupivacaine hydrochloride in rats,‖‖ confirming absence of rapid burst release and uptake *in vivo*. Subsequent quantitative studies were confined to bupivacaine doses ranging from 50 to 450 mg/kg. In this dose range, no systemic toxicity, judged by somnolence, convulsions, or death was observed in any animal (n > 200 animals). In all cases, no systemic analgesia or sensory or motor blockade was observed in the contralateral leg.

Control groups that were injected with either placebo microspheres or with microspheres containing 0.05% w/w dexamethasone alone showed no sensory or motor block. The time course of sensory and motor blockade in groups of rats injected with 150 mg/kg bupivacaine in 65/35 PLGA microspheres that contained 0%, 0.005%, or 0.05% dexamethasone, respectively, is shown in figure 6. The mean durations of the blocks were 8, 50, and 170 h, respectively. Sensory and motor block were prolonged in a similar manner in proportion to the percentage of dexamethasone incorporated over this dose range. There was no evidence of significant separation between durations of sensory and motor blockade for any formulation.

The duration of blockade produced by a series of formulations at a dose of 150 mg/kg bupivacaine containing 0.05% w/w dexamethasone with drug loadings ranging from 45% to 75% w/w is shown in table 3. No burst release was observed *in vitro* over this range of loadings (data not shown). It is apparent that block duration is not greatly influenced by percent drug con-

Table 2. The Duration of Sensory Block for 75/25 PLGA and PLA Formulations Tested *In Vivo*

Polymer Composition	% Dexamethasone (weight/weight) (microspheres)	Duration of Block (h)*
75/25 PLGA	0.05	70 ± 18.0
75/25 PLGA	0	3.0 ± 1.0
PLA	0.05	103.0 ± 14.0
PLA	0	6.0 ± 3.0

* Time for the mean duration of latency to fall from 12 to 7 s.

tent of the microspheres over the 55–75% loading range.

Plasma bupivacaine concentrations after sciatic block injection of 150 mg/kg bupivacaine in 65/35 PLGA with and without 0.05% w/w dexamethasone are shown in table 4. In all cases, plasma concentrations were below 0.5 μg/ml, which is at least fourfold below the threshold for central nervous system toxicity in humans or rats.

Discussion

An injectable local anesthetic preparation has been developed that provides prolonged blockade of a peripheral nerve for longer than 5 days with a wide margin of safety regarding systemic toxicity, in rats. We believe this to be the longest duration, nondestructive, single injection method of local anesthetic blockade to date. Alternative delivery systems for prolonged local anesthetic blockade under investigation include liposomes[19–21] and lecithin-coated microcrystals.[22] Previous reports using liposomes have produced block durations of 2–10 h. Lecithin-coated tetracaine microcrystals give block durations ranging up to 43 h. Durations longer than 2 days would be useful for both acute and chronic pain applications.

Most drugs used previously for controlled release are of high potency; for example, the Norplant birth control system (Wyeth-Ayerst, Philadelphia, PA) releases the synthetic progestin levonorgestrel at an estimated rate of 85 μg per day for the first 9 months. In a previous PLGA microsphere-based release system, the Lupron depot delivers over roughly 1 month a total dose of 3.75 mg of the gonadotropin releasing hormone leuprolide acetate in a loading of approximately 9% w/w.[23] In contrast to drugs used in previous controlled-release delivery systems, all clinically available local anesthetics require considerably higher doses for clinical use. Typical bupivacaine infusion rates for continuous blockade of peripheral nerves or continuous epidural blockade for acute or chronic pain in adult humans range from 5–30 mg/h or 100–600 mg/day.[24,25] To deliver adequate doses of bupivacaine for prolonged blockade, it was necessary to develop a microsphere formulation that permitted much higher percent loadings, up to 75%, which is, to the best of our knowledge, a loading higher than any used in previous clinical application.

The formulations reported here all involve well-studied drugs in a well-studied vehicle. Lactic-glycolic

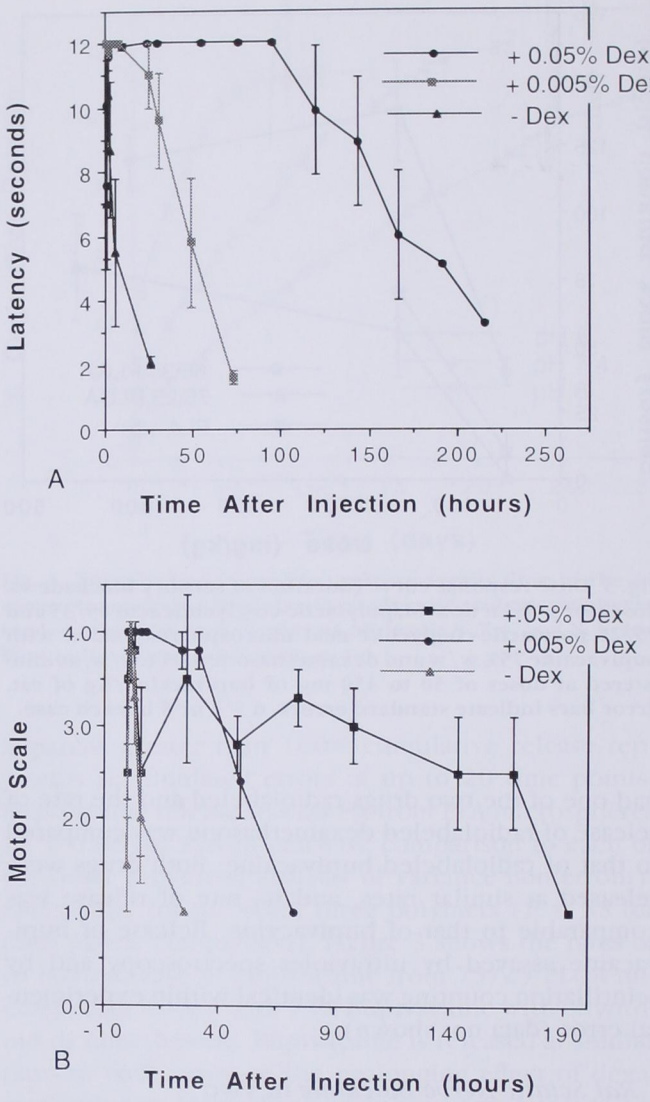

Fig. 6. Comparison of (*A*) the duration of latency *versus* time and (*B*) the motor scale *versus* time for 75% bupivacaine loaded 65/35 polylactic-co-glycolic acid microspheres containing 0.05%, 0.005%, and 0% dexamethasone. Error bars indicate standard errors, n = 10.

polymers have been used widely in clinical use as drug delivery systems with good biocompatability.[10] They are available in high purity. Both monomers are metabolized *via* the Krebs cycle.[9] Specific modifications of previous formulation methods were developed to eliminate formation of bupivacaine crystals external to the microspheres. Examination of the final preparations by environmental scanning electron microscopy showed no evidence of porosity and a smooth, uniform composition over the scale of microns. Microspheres stored in a desiccator under vacuum showed repro-

Table 3. The Duration of Sensory Block for 65/35 PLGA Microspheres Loaded with 0.05% weight/weight Dexamethasone and Varying Amounts of Bupivacaine Free Base and Corresponding Mass Median Diameter

Medisorb Batch No.	% Bupivacaine Drug Loading (weight/weight)	Mass Median Diameter (μm)	Duration of Sensory Block (h)*
316	76.7	120	145
312	65.0	92	155
319	55.0	84	173
327	46.0	70	55

* Time for the mean duration of latency to fall from 12 to 7 s.

ducible *in vitro* and *in vivo* results for up to 18 months after formulation. Once microspheres are suspended in an aqueous medium, it is advisable to use them promptly to minimize the initial aqueous concentration of free drug at the time of injection.

Bupivacaine was released from these microspheres in a controlled manner *in vitro*. In our most studied formulation, 65/35 PLGA, roughly 20% of the bupivacaine was released in the first 24 h, and approximately 7% released daily thereafter up to approximately day 10. More than 90% of the bupivacaine was released by day 15 for all formulations. Controlled release *in vivo* is further confirmed by the lack of observed toxicity even with doses of 600 mg/kg, more than 30–150 times the convulsant dose for aqueous bupivacaine hydrochloride. We would estimate from the lack of convulsions that no more than 3–5% of the bupivacaine is released within the first hour after injection *in vivo*.

The aqueous solubility of bupivacaine depends greatly on its degree of ionization, which is in turn a function of *p*H. The pKa of bupivacaine is 8.1.[3] The solubility of bupivacaine (largely as the hydrochloride) in water at *p*H 1.8–6 is 40–50 mg/ml, whereas the solubility of the unionized form at *p*H 8–12 is reported to range from 0.15–0.25 mg/ml.[26] As is shown in figure 4, the pH level of the buffer used has a strong effect on the release rates of the bupivacaine from 75/25 PLGA. As expected, at *p*H 6 the rate of release was faster than that observed at *p*H 7.4 and 8, due to the increased aqueous solubility of the unionized form of bupivacaine at *p*H 6.

Dexamethasone coincorporated into bupivacaine-polymer microspheres prolonged sensory and motor block up to 8–13-fold relative to bupivacaine-polymer microspheres without dexamethasone. Although there are anecdotal descriptions of prolongation of analgesia in patients who received local anesthetic blocks in certain clinical situations, we could not identify any previous studies showing prolongation or intensification of sensory or motor blockade by glucocorticoids.[27] Analgesia from dexamethasone is commonly ascribed to an antiinflammatory effect, suppressing generation of nociceptive mediator compounds, rather than to an intensification of conduction blockade.

Inclusion of dexamethasone in the microspheres did not substantially effect the *in vitro* release rate of bupivacaine. The prolongation of block observed *in vivo* in the presence of dexamethasone was not predicted by the patterns of *in vitro* release. Several alternative explanations may account for the differences between *in vitro* release rates and *in vivo* duration of action observed behaviorally. First, the pharmacokinetics of drug release from the microspheres *in vivo* may differ from those in *in vitro* conditions, and dexamethasone may slow drug release *in vivo* by preventing encapsulation or inflammation. Second, dexamethasone could conceivably alter the kinetics of bupivacaine entry into nerve or the kinetics of bupivacaine clearance from the site. Third, dexamethasone's effect may be

Table 4. The Time between Microsphere Injection and Removal of Blood, the Bupivacaine Levels in the Plasma of Rats that Received 150 mg/kg of 65/35 PLGA Microspheres Loaded with 75% weight/weight Bupivacaine and Either 0.05% Dexamethasone or No Dexamethasone

Time between Injection and Extraction of Blood (days)	Bupivacaine Plasma Concentrations (μg/ml)	
	Rats that Received 150 mg/kg of 65/35 PLGA Loaded with 75% Bupivacaine and 0.05% Dexamethasone	Rats that Received 150 mg/kg of 65/35 PLGA Loaded with 75% Bupivacaine
1	0.217 ± 0.026	0.290 ± 0.021
2	0.236 ± 0.099	0.232 ± 0.027
7	0.030 ± 0.015	<0.005*
24.5	0.015 ± 0.008	0.014 ± 0.009

* The lower detection limit is 0.005 μg/ml.

independent of bupivacaine release rates, *i.e.,* a pharmacodynamic interaction that intensifies nerve blockade at any given intraneural bupivacaine concentration. Fourth, dexamethasone's block-prolonging effect may be related to inhibition of local anesthetic tachyphylaxis. Investigations to discriminate among these and other alternatives are in progress and will be reported separately.

In summary, we have developed an injectable local anesthetic preparation that provides 2–5-day blockade of the sciatic nerves of rats *in vivo.* This may form the basis of a clinically useful method for providing prolonged regional anesthesia or analgesia, particularly in locations where protective sensation is not required. For example, 5-day intercostal blocks for lateral thoracotomy or 5-day ilioinguinal/iliohypogastric blocks for inguinal hernia repair may be convenient methods for postoperative analgesia.

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