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Prolonged Intercostal Nerve Blockade in Sheep Using Controlled-release of Bupivacaine and Dexamethasone from Polymer Microspheres

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Background: **Previous work from the authors' group characterized a prolonged percutaneous blockade of the sciatic nerve in rats using bupivacaine–dexamethasone microspheres. The goals of the current study are to examine the (1) efficacy of bupivacaine microspheres with and without dexamethasone for intercostal blockade in sheep; (2) scaling of dose and duration with a 100-fold increase in body size from rats to sheep; (3) local toxicity and adverse systemic reactions to bupivacaine microspheres with and without dexamethasone.**

Methods: **Intercostal blocks were performed percutaneously in sedated sheep. Sensory blockade was measured at repeated time points by absent flinch response to skin pinch. Plasma bupivacaine concentrations were measured using high performance liquid chromatography. Chest wall specimens were examined by light microscopy.**

Results: **The duration of intercostal blockade increased with bupivacaine dose for animals receiving from 8 to 80 mg/kg of microspheres with and without dexamethasone. At each dose, microspheres containing dexamethasone had a longer duration of block than microspheres without dexamethasone. From 8 to 80 mg/kg, the mean duration of block with bupivacaine–dexamethasone microspheres increased from 4 to 13 days. Plasma concentrations of bupivacaine remained 10-fold below the convulsive EC_{50} concentration for sheep. Chest wall histology showed a significant granulomatous reaction around bupivacaine microspheres but not around bupivacaine–dexamethasone microspheres.**

Conclusions: **A single administration of bupivacaine–dexamethasone microspheres produces an effective chest wall analgesia of several days' duration. This may prove useful clinically for thoracic surgery or trauma. (Key words: Local; nerve block; sustained-release.)**

LOCAL anesthetics are used to provide postoperative analgesia. Single injections of currently available local anesthetics, such as bupivacaine, rarely provide analgesia for more than 6–12 h. Various approaches have been tried to prolong local anesthetic action; indwelling catheters are useful, particularly in the epidural space, but they are inconvenient to maintain in many other sites. Alternative methods to prolong block duration are under investigation,[1–3] but none is currently in clinical use. Neurolytic techniques are available for prolonged blockade, but because of their capacity to produce deficits and new forms of pain, their use is limited in nonterminally ill patients. Even cryoanalgesia, which is touted as less injurious to nerves, may produce neuritis in many patients.

Our group has developed a series of bioerodable sustained release polymer local anesthetic pellets and microspheres. Implantation of the pellets and injection of the microspheres have been shown to produce sensory and motor blockade of the rat sciatic nerve for periods ranging from 6 h to 5 days, depending on dose and composition.[4–7] In the course of these studies, it was found that the incorporation of dexamethasone not only decreased the inflammatory response to pellets or microspheres, but also prolonged the blockade duration. The block-prolonging effect of dexamethasone was shared by a number of other corticosteroids,[8] with the rank-order of block-prolonging effect increasing with the rank-order of anti-inflammatory potency. Non-hormonal sterols, including cholesterol, or other steroidal hormones, including estrogen, progesterone, and testosterone, as well as the non-steroidal anti-inflammatory drug (NSAID) ketorolac were ineffective in prolonging blockade.

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In contemplating future clinical application of this delivery system, it is pertinent to evaluate how the effective dose scales with body size and whether any adverse effects occur at a higher dose level. Previous work in rats and isolated nerve preparations suggest that the effective dose to block a nerve varies only weakly with body size.[9,10] Therefore, it is important to test these preparations in a species with a size more comparable to humans.

Intercostal blockade has been used clinically in the management of postthoracotomy pain and pain caused by rib fractures. The usefulness of intercostal blockade is currently limited greatly by the short duration of existing local anesthetic preparations. Because of the expected clinical importance of long-duration intercostal blockade, in the present study, we examined efficacy, local tissue reactions, pharmacokinetics, and the risk of systemic toxicity after percutaneous intercostal blocks in sheep.

Materials and Methods

Microsphere Formulation

Poly-lactic-glycolic-acid microspheres (Medisorb, Inc., Cincinnati, OH) consisted of a 65:35 lactic acid-to-glycolic acid weight ratio. Microspheres loaded with bupivacaine (Lot 386I) were analyzed and found to contain 75 ± 2% w/w bupivacaine. Microspheres loaded with bupivacaine and dexamethasone (Lot 384I) were analyzed and found to contain 75 ± 2% w/w bupivacaine and 0.05% dexamethasone. The suspending medium for injection consisted of 0.5% w/v sodium carboxymethylcellulose (low viscosity), 0.1% w/v Tween 80, and 0.18% methyl paraben pH = 6.5 (Medisorb, Inc). Microspheres were suspended in this injection vehicle in a volume of 2–3 ml per rib. This vehicle is used clinically for injection of other depot medications, including Lupron® depot (TAP Pharmaceutical Company, North Chicago, IL).

Drug doses were calculated on a mg/kg basis because aqueous local anesthetic doses are usually calculated on the basis of body weight. Microsphere doses used were 1.6, 8, 20, 40, and 80 mg/kg of bupivacaine microspheres or bupivacaine–dexamethasone microspheres. A dose of 1.6 mg/kg was approximately equal to 10 mg microspheres per nerve. A dose of 80 mg/kg yielded approximately 470 mg of microspheres, containing 352.5 mg of bupivacaine, per nerve.

Animals

The animal protocol for the following experiments was approved by the Animal Care Committees of both Children's Hospital and the Biodevelopment Laboratories Inc., Cambridge, Massachusetts, where the experiments were carried out. Animals used in this study were 21 female sheep (Dorset crossbreed, Parson's Farm, Hadley, MA) ranging in weight from 21 to 52 kg and in age from 5 months to 1 yr.

Aqueous Bupivacaine Injections

Two naïve animals received intercostal injections for five nerves with 3 ml of 0.25% aqueous bupivacaine per nerve to establish a baseline on which microsphere performance could be assessed. Each animal was injected first on the left side of its thorax and then on the right in a subsequent experiment. One animal was injected twice, and the other for a total of four times, alternating sides each time. At least 4 days elapsed between injections for each animal.

Technique of Percutaneous Intercostal Blockade

The dose of microspheres was divided into five equal aliquots because five intercostal nerves were to be blocked in each sheep. Immediately before injection, syringes were mixed at high speed for 1–2 min. The suspended microspheres were injected using 20-gauge needles. Each dosage was tested in a group of at least five sheep. In each experiment every sheep received five injections for five intercostal nerves on either the left or right side of its thorax. After an experiment, animals were rested for a minimum of 3 weeks to allow any residual drug to clear the body. Most sheep received multiple injections of microspheres, alternating in successive experiments between the left and right sides. Injections on the same side were given at least 6 weeks apart, and no more than three times overall. For histologic studies, animals were used that had never been injected previously.

Sheep were sedated with 0.25–0.35 mg/kg xylazine (Miles Inc., Kansas City, MO) by intramuscular injection 20 min before intercostal block injections. After sedation, animals were removed from their pen and brought to a procedure room. They were placed on the operating table in lateral decubitus position with extremities lightly restrained by a second investigator. Wool was shaved in an area on the left or right side of the thorax from the vertebra to the sternum and from the caudal margin of the scapula to the caudal border of the thirteenth rib. Five nerves was the maximum number of

nerves not obscured by the scapula and therefore accessible to percutaneous injection. The intercostal nerve of the thirteenth rib was not injected because of overlapping innervation with the first lumbar nerve. The area was then prepared for injections under aseptic condition. The eighth to twelfth intercostal spaces were identified by surface landmarks, and needles were placed 10 cm lateral to the dorsal midline. The ventral and dorsal rami of the intercostal nerves in sheep diverge more centrally than in humans, requiring superficial and deeper injection at the caudal edge of each rib to anesthetize both branches.[11,12] The cutaneous branches of these rami innervate the skin from the dorsal midline, over the lateral and ventral chest wall down to the ventral midline. After injections, the animals were returned to their quarters and tested at hourly intervals on the same day and then twice daily on subsequent days until the nerve blockade resolved.

Sensory Testing

All observations were made by a single blinded investigator [C.D.] to minimize variation in technique. Sheep were tested in their home pens. An assistant lightly restrained the animal. Sheep were acclimatized to this sort of light restraint for 10 days before the onset of testing. Skin sensitivity was tested by the investigator with curved blunt forceps (tip width, 0.8 mm) applied until a response was observed, but no longer than 2 s. A normal response to this stimulus is a reflex contraction of the subcutaneous truncal musculature at the point of stimulation or a withdrawal reaction. Preliminary experiments showed that a painful stimulus lasting 2 s produced a response in every case, unless there was complete nerve blockade. Two seconds were timed with a stopwatch. A skin fold was squeezed with a force that would promptly cause a reaction on the contralateral side. A pinch test was done on the opposite side of the thorax 1 min before every sensory testing of the injected side.

Responses were coded as: [1] complete block, defined as no flinch or muscle contraction and no gross bodily movements after forceps application for 2 s, [2] partial block, defined as delayed or decreased flinch and no gross bodily movement, or [3] unblocked, defined as any combination of fast twitches of the chest wall, sudden postural movements, foot stomping, or escape movements. Corresponding sites on the contralateral chest wall were tested repeatedly to control for systemic effects of local anesthetics, residual sedation, stress-induced analgesia, or other behavioral factors that could potentially confound these measurements. For purposes of analysis, we quantitated only the area of dense blockade, as defined previously.

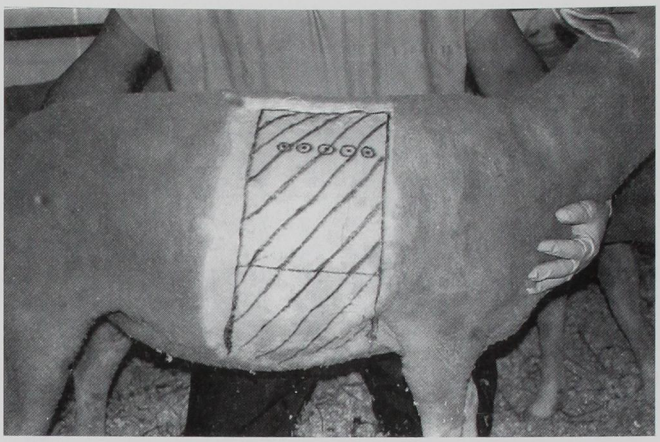

Fig. 1. Areas of cutaneous analgesia and anesthesia are marked on a sheep's thorax. Thoracic nerves have a straight segmental anatomy, and each nerve innervates a strip of skin from the dorsum to the sternum. Dots signal the five intercostal injection sites; striped rectangular area marks the area of anesthesia around the injected sites along five thoracic nerves.

Insensitive regions were marked directly on the animals' skin and quantified in cm^2 at the end of each test (fig. 1). We recorded the absolute area blocked at each time, the time to 50% reduction in initial area of blockade, and the time for complete recovery of sensation in the entire area of initial blockade.

Animals were tested for intercostal sensory blockade starting 1 h after completion of the last injection. However, because the sedative effect of xylazine can last up to 2 h, data are only included for time points beginning 3 h after injection. At that time, the animals were fully alert and responded promptly to a contralateral pinch. A contralateral pinch test was done 1 min before every sensory testing of the injected side to ensure that delayed or decreased responses caused by residual sedation were not falsely attributed to true intercostal blockade.

Blood Collection and Analysis

Venous blood samples were collected from the jugular vein on the day of the microsphere injection at 3–6 h after injection, 24 h later, and then every other day for a maximum of 4 weeks after injection. Blood was drawn into EDTA tubes, centrifuged, the plasma frozen, and subsequently assayed for bupivacaine. Plasma bupivacaine concentrations were determined by reversed phase high performance liquid chromatography (HPLC) modified to increase sensitivity. After addition of pentylcaine as the internal standard, plasma samples were

Table 1. Duration (h) of Microsphere Treatment at Each Intercostal Injection Site at Time of Death

	Animal 1		Animal 2		Animal 3		Animal 4		Animal 5		Animal 6	
	L/ BD	R/ BD	L/ BD	R/ BD	L/ B	R/ B	L/ B	R/ BD	L/ B	R/ BD	L/ B	R/ BD
7. ICS	7	7	7	7	7	7	7	7	48	48	96	96
8. ICS	24	24	24	24	24	24	7	7	48	48	96	96
9. ICS	48	48	48	48	48	48	7	7	48	48	96	96
10. ICS	72	72	72	72	72	72	7	7	48	48	96	96
11. ICS	96	96	96	96	96	96	7	7	48	48	96	96

B = bupivacaine; BD = bupivacaine/dexamethasone; ICS = intercostal space.

added to a solid phase extraction cartridge, rinsed, and then eluted with methylene chloride and isopropanol. After evaporation of the organic phase, the residue was reconstituted in mobile phase (65% 0.04M K2HPO4 and 35% acetonitrile) and injected onto a C-18 column with ultraviolet detection at 205-nm wavelength. The assay was linear over the range of 5 to 500 ng/ml with interassay between day coefficients of variation of 10% or less over this entire range of concentrations.

Histology

Six naïve animals were injected on both sides with a dose of 20 mg/kg bupivacaine microspheres or bupivacaine-dexamethasone microspheres. Two animals received only bupivacaine-dexamethasone microspheres, and one animal received only bupivacaine microspheres. The other three animals were injected at several different timepoints with bupivacaine microspheres on the left side and bupivacaine-dexamethasone microspheres on the right side (table 1).

Injections at several different time points for animals #1-3 allowed animals to serve as their own controls. Animals #4-6 were killed at three time points: at 7 h, 2 days, and 4 days after injection, respectively; these time points were chosen on the basis of pilot data from rats, which showed that the progression of inflammation could be distinctly observed at these time points. The six sheep were killed with 10 ml of Euthanasia-5 solution (Henry Schein; Port Washington, NY) intravenously. The rib cage around the microsphere injection sites was resected in one large piece and placed in 10% buffered formalin. Sections of intercostal muscles containing the respective injection sites were later removed from the fixed chest wall, imbedded in paraffin, sectioned (4-μm thickness) and processed using hematoxylin and eosin staining. Sections were coded numerically for blinding and then were examined for the presence of inflammatory cells using light microscopy. Different classes of inflammatory cells were scored blindly at 400× magnification for the time points: 7 h, day 2, and day 4. The scoring scale ranged from 1 to 4. Zero cells per field were scored as "0," 0-10 cells per field were scored as "1," 10-100 cells per field were scored as "2," 100-300 cells per field were scored as "3," and more than 300 cells were scored as "4." In addition to the histopathology of muscle, samples of intercostal nerves were fixed in formaldehyde and examined by light microscopy for signs of nerve injury. These nerves were obtained from three sheep that had been dosed multiple times with bupivacaine microspheres with and without dexamethasone at 40-80 mg/kg.

Statistical Analysis

Parametric data (initial blocked area, duration of blockade) were analyzed by regression analysis ($y = A + Bx$), analysis of variance, and Bonferroni corrected Student's one-sample t test or two-sample t tests. For *post hoc* comparison between groups at a particular time, the Student's t test with the Bonferroni correction was used. Histologic scores (1-4) were ranked and analyzed with a Mann-Whitney U test. Significance for all statistics was measured at a level of $P < 0.05$.

Results

Aqueous Bupivacaine

Intercostal injections with 0.25% bupivacaine produced blockade that disappeared completely in 12 ± 1.1 h (SD; fig. 2A). Blocked area regressed to 50% of maximum area in 8.5 ± 1.5 h.

Intercostal Sensory Blockade Using Bupivacaine Microspheres [without Dexamethasone]

Intercostal block injections with aqueous bupivacaine and with microspheres produced an area of chest wall hypesthesia, as outlined in figure 1. The area blocked and duration of blockade did not increase significantly with increasing doses of bupivacaine microspheres (fig. 2). Sheep receiving 1.6 mg/kg of bupivacaine microspheres had no area of complete blockade of the skin of their lateral thorax. Figure 2 compares the area of the anesthetized chest wall for different dosages of bupivacaine microspheres (*A*) and bupivacaine-dexamethasone microspheres (*B*), respectively. It is noteworthy that despite the increasing dosages of bupivacaine mi-

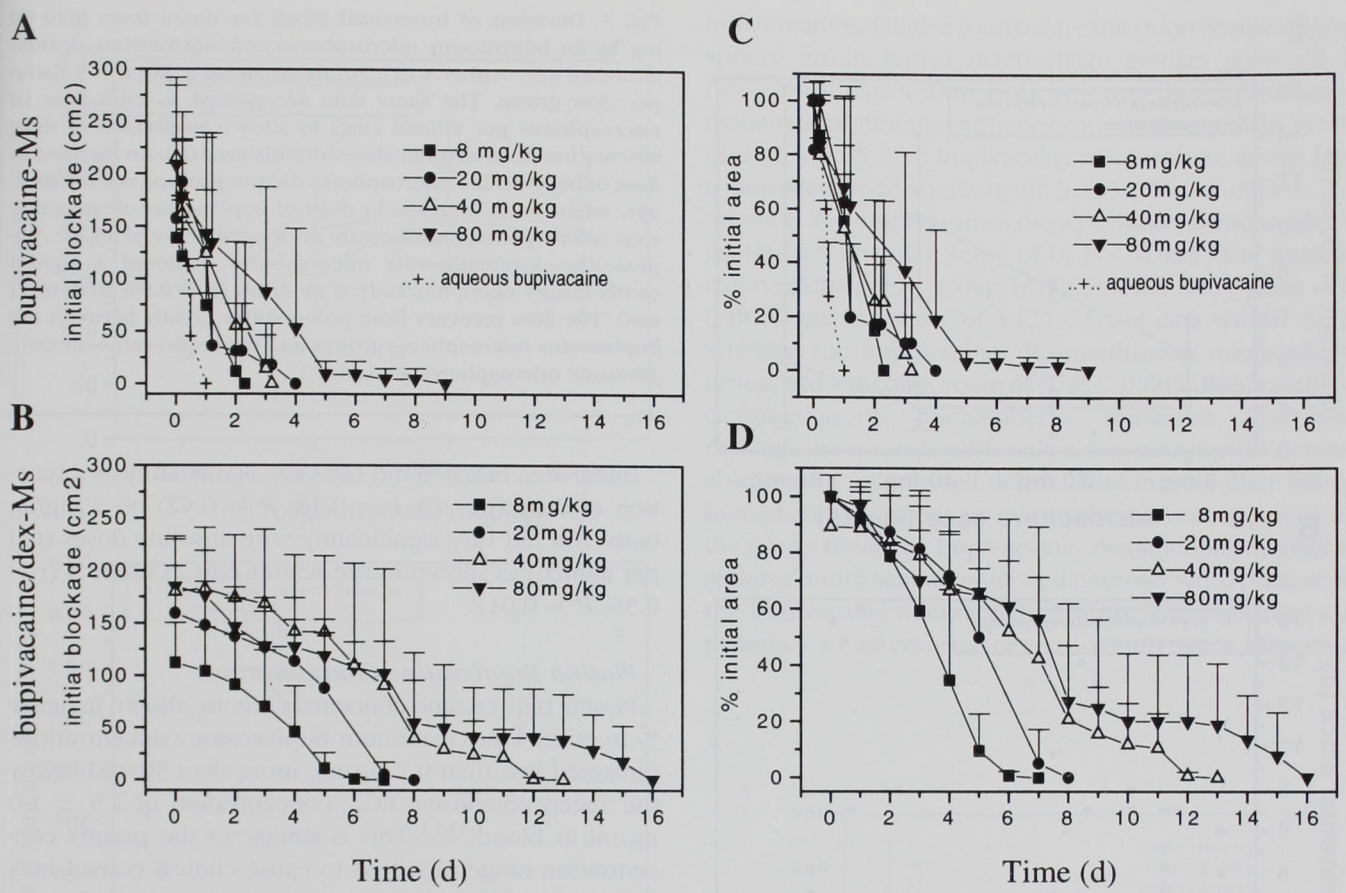

Fig. 2. Area of skin anesthesia in cm² plotted against time for intercostal nerve blockades with bupivacaine microspheres (*A*), and bupivacaine–dexamethasone microspheres (*B*) at dosages ranging from 8 to 80 mg/kg. *C* and *D* show the same data normalized (percentage) for easier comparison of drug efficacy; bupivacaine microspheres (*C*), bupivacaine–dexamethasone microspheres (*D*). Points are mean ± SD; n = 5 sheep per dose-group.

crospheres, the mean duration of block remains nearly constant at around 48 h for dosages of 8–40 mg/kg. An increase in dosage of bupivacaine microspheres did not significantly increase the time to complete recovery of blockade (r = 0.4; $P = 0.06$; fig. 3A) or the time to 50% reduction of initial block area (fig. 3C). The area of initial block did not vary significantly with microsphere dose for the range studied (8–80 mg/kg) for bupivacaine microspheres, as shown in figure 4A (r = 0.2, $P = 0.29$). Absolute dosages given in a dosing group (mg/kg) varied because of different weights of the animals within the group. Size of initial area blocked (bupivacaine microsphere, r = 0.17, $P = 0.45$) or duration of block (r = −0.3, $P = 0.41$) also did not vary significantly with absolute doses (mg per animal), as shown in the scatter plots in figures 3 and 4. The absolute doses were plotted to allow a comparison of results based on amount of anesthetic delivered to each animal and therefore to each intercostal nerve. At all doses, bupivacaine microspheres produced virtually no block beyond 4 days.

Intercostal Sensory Blockade Using Bupivacaine-Dexamethasone Microspheres

The minimum dose required to produce detectable blockade was 8 mg/kg [approximately 37 mg/nerve]. At doses from 8 to 80 mg/kg, bupivacaine-dexamethasone microspheres produced more prolonged blockade (both as time to complete recovery and time to 50% reduction in blocked area) than bupivacaine microspheres when compared for each dose level (Student's *t* test with Bonferroni correction; $P < 0.01$ for each comparison). The effect of dexamethasone on block duration is depicted in figure 2; comparing panels A and B. Figures 2C and 2D, respectively, show the same data normalized to initial area blocked. The dependence of block duration of block on dose was significant (r = 0.73, $P = 0.0002$).

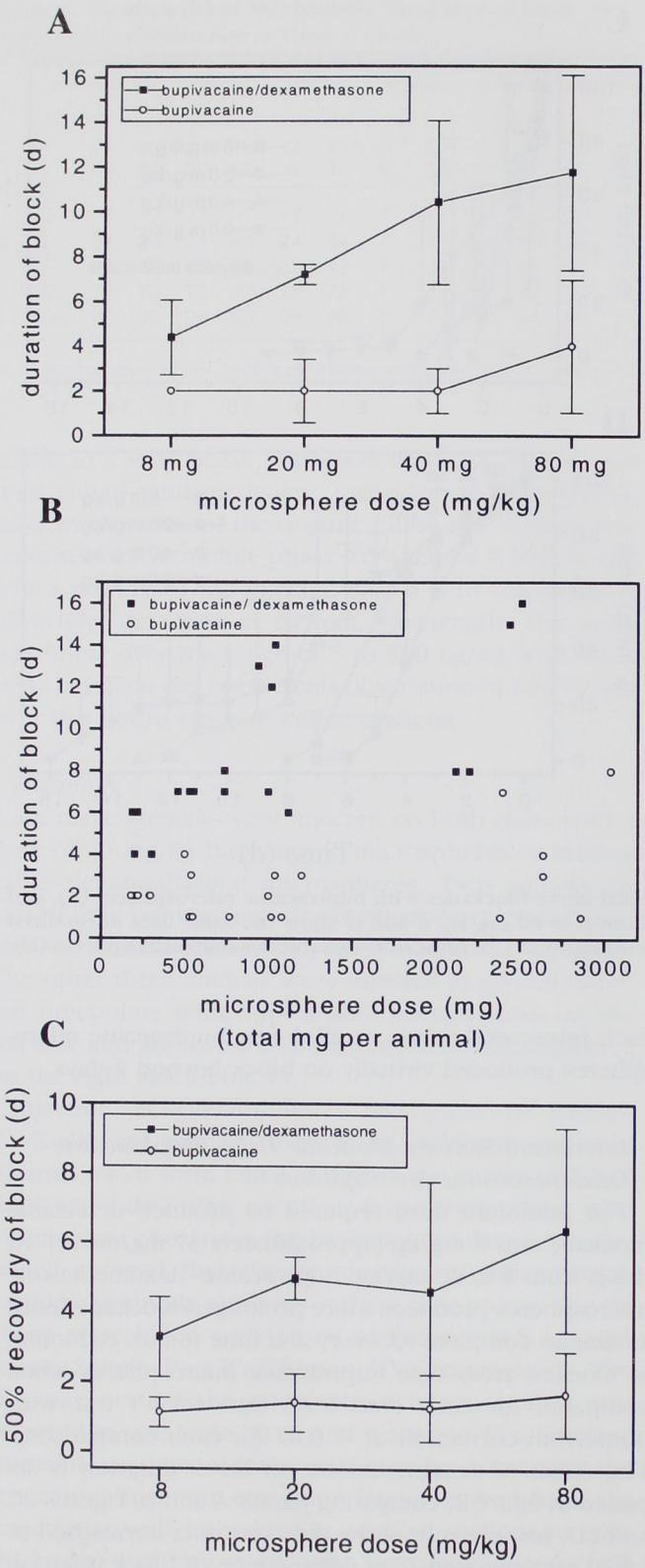

Fig. 3. Duration of intercostal block for doses from 8 to 80 mg/kg for bupivacaine microspheres and bupivacaine–dexamethasone microspheres (*A*). Points are mean ± SD; n = 5 sheep per dose-group. The same data are plotted as total dose of microspheres per animal (mg) to allow comparison of drug efficacy based on absolute dose administered (*B*). An increase in dose of bupivacaine microspheres did not prolong nerve blockade, whereas an increase in dose of bupivacaine–dexamethasone microspheres significantly prolonged nerve blockade. Bupivacaine–dexamethasone microspheres achieved a significantly longer nerve blockade at all doses ($P < 0.05$; Student's *t* test). The 50% recovery time points differ greatly between the bupivacaine microspheres groups and the bupivacaine–dexamethasone microspheres groups (*C*).

Initial area blocked did not vary significantly as a function of dose (fig. 4B; r = 0.18, $P = 0.42$) on a mg/kg basis, but did vary significantly with absolute doses (mg per animal) as shown in the scatter plot in figure 4 (r = 0.55, $P = 0.01$).

Plasma Bupivacaine Concentrations

Plasma bupivacaine concentrations are shown in figure 5. In every case, maximum bupivacaine concentrations remained less than 0.2 μg/ml, more than 30-fold below the sheep convulsive EC_{50} concentration of 7.5 ± 1.0 μg/ml in blood.[13,14] This is similar to the plasma concentration range observed to cause clinical convulsions and cardiac arrhythmias in humans. No animal showed signs of myoclonus, convulsions, or prolonged sedation.

Histology

Macroscopically, the injection sites in the bupivacaine microspheres group appeared as "capsules" of bupivacaine microspheres, whereas the bupivacaine-dexamethasone microspheres could be found interspersed as "sheets" in normal tissue. Figure 6 shows hematoxylin and eosin-stained histologic sections from sheep intercostal muscle after injection with either bupivacaine microspheres or bupivacaine-dexamethasone microspheres. Histology is shown from days 0, 2, 4 (fig. 6A–C) after injection for the bupivacaine group, and days 0, 2, 4 (fig. 6D–F) for the bupivacaine-dexamethasone group.

In the animals treated with bupivacaine microspheres, histologic sections revealed individual muscle bundles pushed apart by edema, whereas there was minimal evidence of edema in the sections from bupivacaine-dexamethasone animals. At 7 h after injection, bupivacaine microspheres caused a strong influx of polymorphonuclear neutrophils (PMN) toward the microspheres with a median score of 3 (25–75% of data at 3) compared

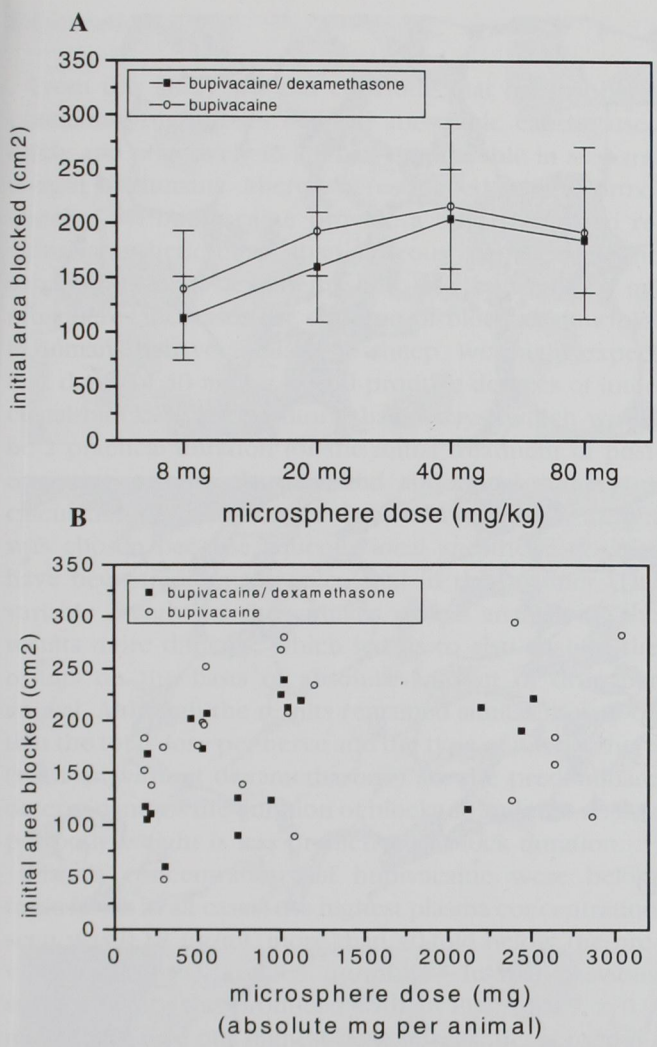

Fig. 4. Comparison of size of the initial area blocked in cm^2 (± SD) for an intercostal block with doses of 8, 20, 40, and 80 mg/kg bupivacaine microspheres and bupivacaine–dexamethasone microspheres (*A*). Points are mean ± SD; n = 5 sheep per dose-group. The same data are plotted as total dose of microspheres per animal (mg) to allow comparison of drug efficacy based on absolute dose administered (*B*). Initial areas blocked correlated significantly with increasing doses of bupivacaine–dexamethasone microspheres, but not bupivacaine microspheres. The two microsphere preparations did not differ with respect to the initial area anesthetized (P = not significant; Student's t test).

with the bupivacaine-dexamethasone microspheres with a median score of 0 (25–75% within 0–1) ($P < 0.005$). Over the next 4 days, the bupivacaine-dexamethasone microspheres in the muscle continued to attract only a small number of cells, resulting in only a mild inflammatory reaction, whereas the bupivacaine microspheres could be found in the center of a granulomatous inflammation. On the fourth day, the bupivacaine microsphere group had a macrophage median score of 2 (25–75% of data within 1–2), whereas the bupivacaine-dexamethasone microsphere group had a median score of 0, $P < 0.05$. The bupivacaine microsphere group had a fibroblast median score of 3 (25–75% of data at 3;) whereas the bupivacaine-dexamethasone microsphere group had a median score of 0, $P < 0.005$. The bupivacaine microsphere group had a foreign body giant cell (FBGC) median score of 3 (25–75% of data within 2–3), whereas the bupivacaine-dexamethasone microsphere group had a median score of 0, $P < 0.005$. Bupivacaine-dexamethasone microspheres remained dispersed through the muscle, with only a few neutrophils detectable among muscle cell debris. Mild muscle fiber injury immediately adjacent to microspheres was apparent in the group receiving bupivacaine-dexamethasone microspheres; more severe injury and necrosis were present in the bupivacaine microsphere groups. The histologic appearance of intercostal nerves, which were dissected

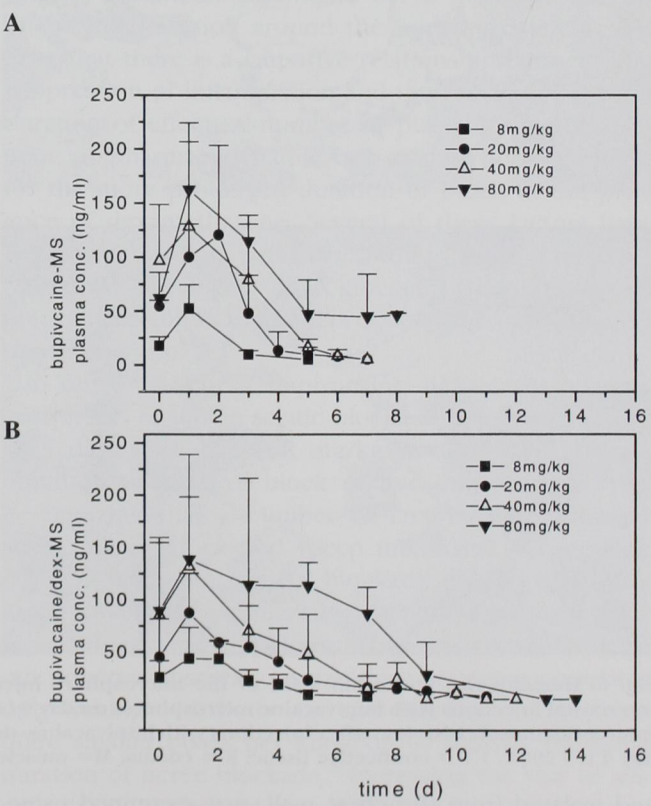

Fig. 5. Plasma concentrations of bupivacaine (ng/ml) (±SD) after intercostal blockade with doses of 8, 20, 40, and 80 mg/kg of bupivacaine microspheres (*A*) and bupivacaine–dexamethasone microspheres (*B*), n = 5.

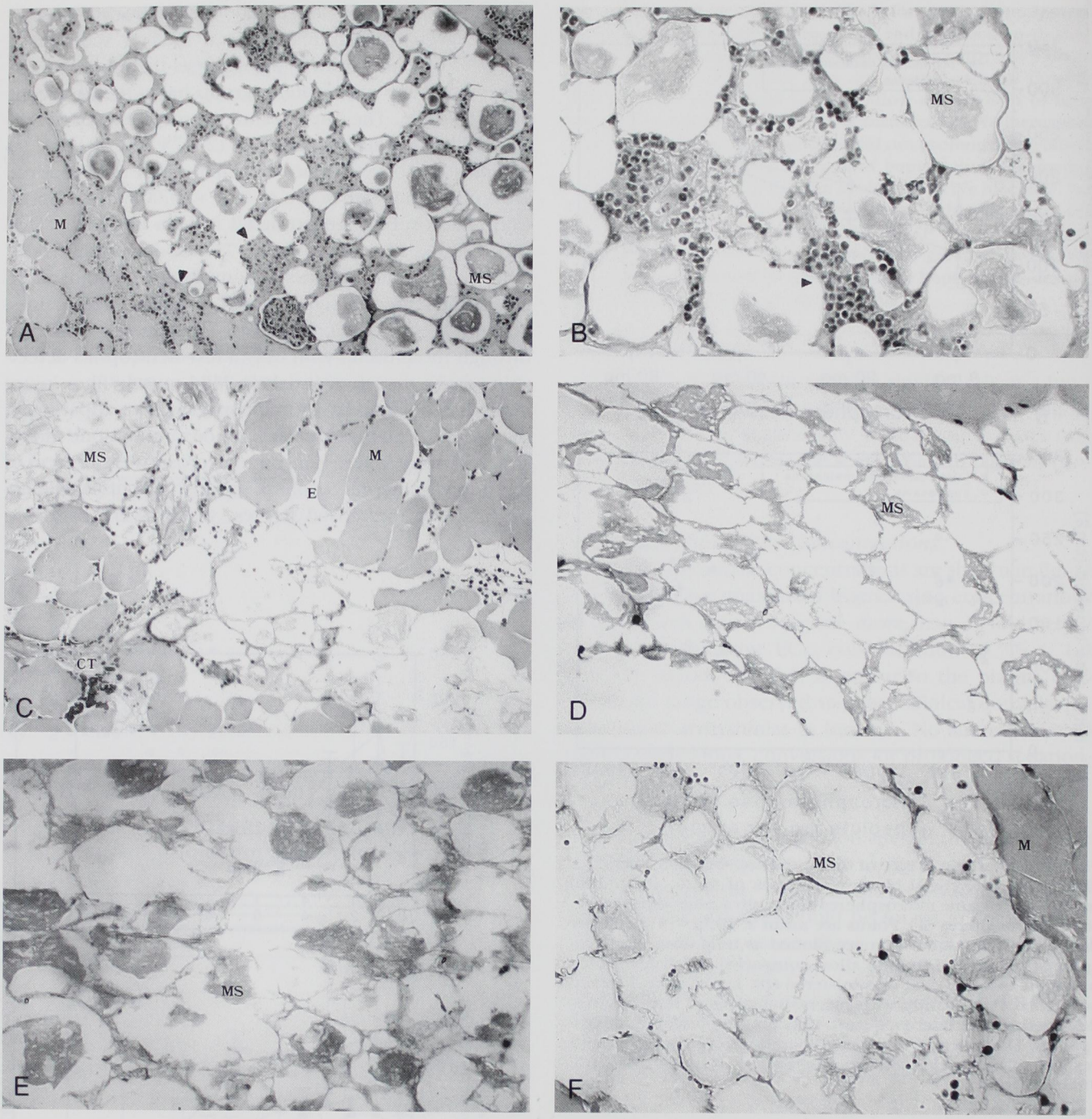

Fig. 6. Hematoxylin and eosin stain of the microsphere injection site in sheep intercostal muscle. *A–C* show histology from intercostal injections with bupivacaine microspheres on day 0 (*A*) 100×, day 2 (*B*) 200×, and day 4 (*C*) 100×, respectively. *D–F* show intercostal muscle histology after injections with bupivacaine–dexamethasone microspheres on day 0 (*D*) 200×, day 2 (*E*) 200×, and day 4 (*F*) 200×. CT = connective tissue; E = edema; M= muscle; MS = microspheres; → = polymorphonuclear leukocytes.

and isolated from the chest wall, was examined using light microscopy. The nerves were fixed in 10% buffered formaldehyde, embedded in araldite, cut into 1-μm thick sections, and stained with p-paraphenylenediamine. No signs of axonal or demyelinating injury after intercostal injections with microspheres could be observed (data not shown, Robert Myers, PhD, oral communication, May 21, 1998).

Discussion

From this study we can conclude that microspheres containing high doses of local anesthetic can be used safely and effectively in animals comparable in size and weight to humans. Microspheres loaded with approximately 75% bupivacaine provide a longer duration regional anesthetic block than aqueous bupivacaine. The incorporation of dexamethasone into bupivacaine microspheres increases the duration of blockade fourfold. If humans behave similarly to sheep, we might expect that doses of 40 mg/kg would produce degrees of intercostal blockade lasting more than 5 days, which would be a practical duration for the initial treatment of postoperative pain in thoracic and abdominal surgery. A calculation of dosage on the basis of mg/kg body-weight was chosen because aqueous local anesthetic dosages have been traditionally calculated in this manner. The variable weight of the animals makes analysis of the results more difficult, which led us to also analyze the results on the basis of absolute amount of drug per animal. Although the results remained similar, it may be that the total dose per nerve and the type of microsphere (with or without dexamethasone) are the predominant determinants of the duration of blockade and that scaling per body weight is less predictive of block duration.

Plasma concentrations of bupivacaine were below toxic levels in all cases; the highest plasma concentration seen was 0.18 μg/ml, more than 40-fold below the previously cited EC_{50} of 7.5 μg/ml.[13,14] In that previous study, toxicity was produced with an ED_{50} of 8.9 ± 0.9 mg/kg. Because our highest dose microspheres used 60 mg/kg of bupivacaine, it is apparent that release of bupivacaine from microspheres is very controlled *in vivo,* and initial burst release in the first 6 h must be less than 5–10% of the total contents of the microspheres. This lack of initial burst release is to be contrasted with the properties of microspheres reported previously by other groups.[15,16]

These data do not address the risk of initial high plasma free-drug concentrations after inadvertent intravenous injection of the microspheres. Studies conducted by Dr. Joseph Tigner and colleagues at Purdue Pharma found no signs of systemic toxicity when dogs received more than 150 mg/kg of bupivacaine in microspheres by direct intravenous bolus injection (J. Tigner, oral communication, August 1995).

The results from intercostal injections with bupivacaine microspheres (with or without dexamethasone) showed no correlation between the initial area blocked and the microsphere dosage or type of microsphere. A dose of 80 mg/kg of bupivacaine–dexamethasone microspheres did not anesthetize a larger area than 8 mg/kg of bupivacaine–dexamethasone microspheres or bupivacaine microspheres. The duration of blockade was not correlated with dosage for bupivacaine microspheres: the difference in recovery times between the highest (80 mg/kg) and lowest (8 mg/kg) dose of bupivacaine microspheres was not significantly different ($P > 0.05$). In contrast, there was a more apparent dose–response for recovery times with bupivacaine–dexamethasone microspheres (20, 40, and 80 mg/kg gave significantly longer durations than 8 mg/kg; and 40 and 80 mg/kg produced significantly longer durations than either 8 or 20 mg/kg). There was no statistical difference in the recovery times between bupivacaine–dexamethasone microspheres in doses of 40 mg/kg compared with 80 mg/kg. It cannot be determined from the currently available data if further increases in dose produce any further prolongation of blockade.

The addition of dexamethasone to bupivacaine microspheres essentially prolonged nerve blockade and reduced inflammation around the injection site. We believe that there is a causative relationship between the suppression of inflammation and the remarkably longer duration of effect. A number of plausible pharmacokinetic or pharmacodynamic factors may be responsible for the more prolonged duration of block in the presence of dexamethasone. Several of these factors have been examined in previous studies.[7,8] An interesting finding is the report that glucocorticoids prolonged block according to the rank order of their anti-inflammatory potencies.[8]

In rats, 150 mg/kg bupivacaine–dexamethasone microspheres results in sciatic blockade lasting an average of 5 days.[7] In sheep, 8 mg/kg resulted in the same duration of effective block of five intercostal nerves. Recognizing that a number of factors may make rat sciatic nerve block and sheep intercostal nerve block different, sheep intercostal blockade appears to require much lower doses in mg/kg per nerve to produce 5-day blocks than rat sciatic blockade. This is consistent with our previous observations that dose per nerve correlates only weakly with body size and that larger animals require smaller doses per kilogram to achieve the same duration of nerve blockade.[6] Increasing the size of animals 100-fold showed that the therapeutic index may become larger with larger animals because the effective dose per nerve scales only weakly with body size.

The differences in efficacy and duration between bu-

pivacaine microspheres with dexamethasone *versus* bupivacaine microspheres without dexamethasone could not be explained solely by the rate of release of bupivacaine from the microspheres because plasma concentrations of bupivacaine were similar in treatment groups at each bupivacaine dose with or without dexamethasone.

We hypothesize that the greater duration of blockade for bupivacaine-dexamethasone microspheres compared with bupivacaine microspheres may be associated in part with dexamethasone's effects in suppressing the inflammatory and foreign body response altering entry of bupivacaine to the intercostal nerves. In an analogous model using rat sciatic nerve blockade, we found recently that bupivacaine microspheres, but not bupivacaine-dexamethasone microspheres, produce local, but not systemic tissue acidosis. In addition, intrasciatic nerve bupivacaine concentrations after injection of bupivacaine microspheres were much lower than those produced by comparable doses of bupivacaine-dexamethasone microspheres. (Dräger C, Benziger D, Gao F, Berde CB, manuscript in preparation). Local tissue acidosis decreases the percentage of released bupivacaine in the neutral form, which penetrates membrane permeability barriers much more efficiently than the protonated form.

A second possible factor may be that in the presence of inflammation, the axonal conduction and impulse-generating properties may be altered,[17,18] and higher intraneural bupivacaine concentrations may be required to produce blockade.

Gross dissection of the injection sites revealed that the bupivacaine microspheres became encapsulated and remained clumped in one area, whereas the bupivacaine-dexamethasone microspheres dispersed freely in the area around the treated nerve and between fascia or inside muscle with a minimal gross inflammatory reaction. These gross observations were confirmed by the histologic examination of the injection sites. Bupivacaine microspheres were rapidly attacked by neutrophils, followed by an invasion of macrophages and foreign body giant cells. The degree of accompanying muscle necrosis was higher in tissue injected with bupivacaine microspheres. However, the amount of bupivacaine was the same in both conditions. The cause for the muscle necrosis might be an intrinsic cytotoxic effect of bupivacaine on muscle[19] and could also be compounded by the mechanical pressure the spheres exert in restricted muscle tissue planes. We believe that the difference in the severity of tissue damage between treatment groups (with and without dexamethasone) may be related in part to the effects of the dexamethasone in suppressing the inflammatory and foreign body response as well as the effects of dexamethasone on the pattern of distribution of the microspheres in tissue planes.[20,21] Bupivacaine microspheres remained congregated in one area in contrast to the well-distributed bupivacaine-dexamethasone microspheres. Bupivacaine microspheres were quickly encapsulated by granulomatous tissue and consequently remained massed at the injection site. In this trapped state, they exert continuous pressure at the same site and irritate the adjacent muscle with a high local concentration of bupivacaine. Capsule formation as a reaction to implanted microspheres in tissue has been reported by several investigators.[22-24] However, when injected subcutaneously or subconjunctivally into guinea pigs, usually only minor inflammation was observed.[25] Most previous animal studies and clinical applications of poly-lactic-glycolic-acid microspheres have involved higher potency drugs and therefore lower percent loading of drug (1%) in the microspheres.[22] In these applications, the inflammatory reactions to PLGA microspheres have been mild. Bupivacaine microspheres without dexamethasone appear to produce more local tissue reaction than "empty" PLGA microspheres, for reasons that remain under investigation.

We conclude that bupivacaine microspheres are a safe and effective tool for producing prolonged intercostal nerve block in a large animal. The incorporation of dexamethasone into bupivacaine microspheres significantly prolongs nerve blockade. Sheep represent a large species comparable with adult humans in both body weight and length of nerves. Therefore, we anticipate these findings will have direct relevance for the application of bupivacaine microspheres in human clinical trials.

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