Prolonged Regional Nerve Blockade by Controlled Release of Local Anesthetic from a Biodegradable Polymer Matrix

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Background: Prolonged nerve blockade is potentially useful in the management of many acute and chronic pain problems. Aside from infusions *via* an indwelling catheter, most currently available nondestructive techniques for prolonging local anesthetic action cannot provide more than 1–2 days of blockade. Bioerodible polymer matrixes have been used to deliver a variety of drugs in patients and animals for periods lasting weeks to years. Previously, dibucaine and bupivacaine were incorporated into copolymers of 1,3 bis(p-carboxyphenoxy) propane-sebacic acid anhydride (1:4), and demonstrated sustained release *in vitro* following incubation of the drug-polymer matrixes in phosphate-buffered solution (pH 7.4, 37° C).

Methods: In the present study, cylindrical pellets made from polymer matrixes incorporated with bupivacaine-HCl were implanted surgically along the sciatic nerves of rats. Neural block was assessed by direct observation of motor skills and by leg-withdrawal latency to a hot surface. Biochemical and histologic examinations were performed 2 weeks after implantation.

Results: Sensory and motor blockade was produced for periods ranging from 2 to 6 days. Contralateral control legs receiving polymer implants without drug showed no block. Blockade was reversible, and animals appeared to recover sensory and motor function normally. Biochemical indexes of nerve and muscle function were indistinguishable from contralateral controls.

Conclusions: This biodegradable polymer system provides a promising new alternative for the delivery of local anesthetics to peripheral nerves to produce prolonged blockade for the management of acute and chronic pain. (Key words: Anesthetics, local: bupivacaine; controlled release; polyanhydride; polymer.)

LOCAL anesthetic blockade of nerves is a mainstay in the management of many forms of acute and chronic pain. Following a single injection, currently available local anesthetics rarely can provide analgesia for longer than 6–12 h, and in many cases, only 4–6 h. Several alternatives are under study for more prolonged delivery, but except for devices that rely on infusion pumps and indwelling catheters, it is unlikely that most methods of prolonging blockade,[1] including liposomes,[2] will be effective for more than 24–48 h. Nerve destructive blocks, including those with phenol, alcohol, heat, or cryoprobes, can produce tissue destruction, unwanted deficits, and new forms of pain.[3] Catheter infusions are awkward or difficult to secure in many locations in the body. Application of a timed-release local anesthetic preparation adjacent to nerves would greatly improve present therapies, including catheter infusions or neurolytic blocks, for providing prolonged regional anesthesia of peripheral nerves in patients with postoperative pain, cancer, nerve injuries, or other conditions requiring chronic pain management.

Sustained release of drugs from biopolymer matrixes has become a useful method of prolonged delivery in a number of contexts. For example, the Norplant birth control system releases progestins over 5 yr. A particularly useful type of biopolymer matrix contains subunits linked by bioerodible polyanhydride bonds. Drug release parallels erosion of inert monomers from matrix layers, analogous to peeling onion skin. Polyanhydride polymer matrixes are used in clinical trials for intra-

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cranial release of antineoplastic drugs in patients with brain tumors.[4,5]

Previously, we have shown the sustained release of local anesthetics dibucaine and bupivacaine from a polymer matrix *in vitro*.[6],§ We now report the first *in vivo* trials,[7] using implants consisting of a biocompatible polymer local anesthetic matrix (PLAM) that biodegrades[8] at the site of implantation.

Methods and Materials

PLAM Implants

Biodegradable PLAM pellets were devised from a copolymer material, 20% poly[bis(p-carboxyphenoxy)] propane anhydride and 80% sebacic acid (molecular weight approximately 65,000),[8] impregnated with crystalline bupivacaine-HCl to release this local anesthetic in a controlled manner. Polymer local anesthetic matrix pellets were made by mixing 150-μm sieved crystals of bupivacaine-HCl with polymer powder[6] in 12% and 20% drug:polymer percentages (by weight). In brief, cylindrical pellets were produced by melting the mixtures in a tuberculin syringe at 115° C in a dry oven for approximately 15 min and then injecting the molten mixture into Teflon tubing (internal diameter 3.2 or 4.8 mm). After cooling, the pellets were cut to specified lengths and weights. Control pellets were made in an identical manner using polymer without drug.

Three sizes of PLAM pellets, loaded to 20% by weight with bupivacaine-HCl, were used as implants to examine dosage effects. Group 1 pellets weighed 50 ± 3 mg (47–53 mg) and were 4.9 ± 0.3 mm long with internal diameter 3.1 ± 0.2 mm. Group 2 pellets weighed 100 ± 5 mg and were 9.8 ± 0.2 mm long with internal diameter 3.1 ± 0.2 mm. Group 3 pellets weighed 125 ± 5 mg and were 6.0 ± 0.1 mm long with internal diameter 4.7 ± 0.2 mm. Pellets were sterilized *via* Γ irradiation for *in vitro* or *in vivo* use. Four different batches of PLAM pellets were used, and similar results were obtained. A more detailed discussion of PLAM implant preparation is given elsewhere.[6]

In Vitro

Bupivacaine PLAM pellets (equal in size to Group 2 pellets) loaded with 20% bupivacaine were immersed

in various volumes (2 ml, 10 ml, 25 ml) of phosphate-buffered saline with 0.1% sodium azide (to inhibit bacterial growth; pH 7.4, 37° C). In addition, 12% pellets were immersed in 10 ml of similar phosphate-buffered saline. Buffer was collected and replaced at 0.5, 2, 8, 16, and 24 h, then once daily thereafter for 3 weeks, and stored at −20° C before high-performance liquid chromatography (HPLC) assay using ultraviolet detection (210 nm).[6] Four bupivacaine standards, ranging 0.2–20 μg, analyzed, on average, after every tenth sample, produced linear response values ($R^2 > 0.995$). The sample concentrations fell in the standard range. The limit of detection was approximately 0.01 μg/ml for standards in water and 0.1 μg/ml for standards in plasma.

PLAM Implantation

For surgery, male rats (150–250 g, Sprague-Dawley) were anesthetized with 50–75 mg/kg pentobarbital intraperitoneally for Groups 1 and 2, whereas Group 3 received halothane (4% in oxygen for induction and 2% for maintenance) to allow faster recovery for behavioral measurements. The shaved skin of the dorsal thigh was incised midway between the hip and the knee. The hamstring muscles were divided with a small hemostat, exposing the dorsal aspect of the sciatic nerve. Under direct vision, polymer pellets could be fitted easily into a large space between muscle layers surrounding the nerve. The space containing the pellets was bathed with 0.5 ml of an antibiotic solution (5,000 units/ml penicillin G sodium and 5,000 μg/ml streptomycin sulfate). The fascia overlaying the hamstrings were reapproximated with a single suture before closing skin with two wound clips.

For all rats, PLAM pellets were implanted surgically along the sciatic nerve in the upper thigh (fig. 1), with drug-containing implants on the experimental side and control (drug-free) implants on the contralateral (control) side.

Nerve Block Tests

Motor block. The rats were behaviorally tested for sensory and motor blockade in a quiet observation room at 24 ± 1° C. Polymer local anesthetic matrix implantation was performed only in rats showing stable baseline hot plate latencies after at least 1 week of testing. In all testing conditions, the experimenter recording the behavior was unaware of the side containing the local anesthetic. To assess motor block, a 4-point scale based on visual observation was devised: (1) normal

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§ Berde CB, Masters DB, Dutta S, Turek T, Langer R: Sustained release of dibucaine from a biodegradable polymer matrix: A potential method for prolonged neural blockade (abstract). ANESTHESIOLOGY 73(suppl):A775, 1990.
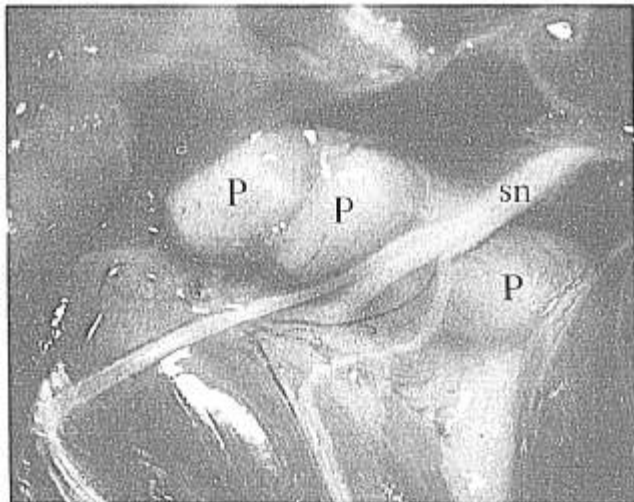
Fig. 1. Photograph of a 10X microscope field depicting the sciatic nerve (sn) at its bifurcation and polymer local anesthetic matrix implants (P) 2 weeks after surgical implantation.

appearance, (2) intact dorsiflexion of foot with an impaired ability to splay toes when elevated by the tail, (3) toes and foot remained plantar flexed with no splaying ability, and (4) loss of dorsiflexion, flexion of toes, and impairment of gait. For graphing clarity, partial motor block equals a score of 2 and dense motor block either 3 or 4.

Sensory block. Using a technique developed in this lab, sensory blockade was measured by the time required for each rat to withdraw its hind paw from a 56° C plate (model 35-D, IITC Life Science Instruments, Woodland Hills, CA). The rats were held with a cloth gently wrapped above their waist to restrain the upper extremities and obstruct vision. The rats were positioned to stand with one hind paw on a hot plate and the other on a room temperature plate. With a computer data collection system (Apple IIe with a footpad switch, Cupertino, CA), latency to withdraw each hind paw from the hot plate was recorded by alternating paws and allowing at least 15 s of recovery between each measurement. If no withdrawal occurred from the hot plate within 15 s for Groups 1 and 2 or 12 s for Group 3, the trial was terminated to prevent injury and the termination time was recorded. Testing ended after five measurements per side, the high and low points were disregarded, and the mean of the remaining three points was calculated for each side. Animals were handled in accordance with institutional, state, and federal guidelines, and all procedures were

approved by animal use and care committees at Children's Hospital and Harvard Medical School.

Necropsy

The animals were killed by carbon dioxide asphyxiation 2 weeks after implantation, approximately 1 week after motor and sensory scores had all returned to pre-surgery baseline levels. *In vitro* approximations predict drug depletion (<5% left) from the polymer matrix by 1 week, corresponding well with the observed block. Thus, the sciatic nerve was receiving less than an effective blocking dose of local anesthetic for approximately 1 week before postmortem analyses. Although *in vitro* release studies predict that very little to no local anesthetic was being released during days 7–14 *in vivo*, tachyphylaxis to persistent local anesthetic exposure for part of this period cannot be ruled out.

Histology

Sections of sciatic nerve approximately 2–3 cm in length, adjacent and proximal to the implants, were preserved in 10% formalin solution (24 mM sodium phosphate, pH 7). Coded sections were embedded in paraffin, stained with hematoxylin and eosin, and examined by light microscopy by a neuropathologist (WK), who was unaware of control *versus* PLAM section identity.

Plasma Analysis

In five rats (250–275 g) anesthetized with ketamine-HCl (100 mg/ml at 1.5 ml/kg intraperitoneally) and xylazine (4 mg/ml at 4 mg/kg intraperitoneally), a silastic catheter was inserted into the right jugular vein, using a procedure developed in the laboratory of Miczek *et al.*[9] Two days after catheter insertion, six Group 1 pellets loaded with 20% bupivacaine (~300 mg total, 60 mg bupivacaine + 240 mg polymer) were implanted next to the sciatic nerve. Blood was withdrawn (0.5 ml) before implantation and 1, 4, 24, 48, 72, and 96 h after PLAM implantation *via* the indwelling central venous cannulae. Plasma proteins were precipitated with an equal volume of HPLC grade methanol (Fischer Scientific, Pittsburgh, PA) centrifuged (10,000 × g), and the supernatant was filtered (0.2-µm nylon syringe type, Rainin, Woburn, MA) before HPLC analysis. The HPLC reliably quantified bupivacaine concentrations in the plasma methanol supernatant as low as 10–50 ng/ml. The bupivacaine standards used for blood plasma analyses were added to plasma aliquots before methanol addition to the sample. The peak matching

the standard bupivacaine peak's retention time was verified in plasma samples by doping with bupivacaine.

Biochemical Assays

Acetylcholine receptor. The gastrocnemius muscle was excised from rats that had received Group 2 implants and assayed for I-125 α-bungarotoxin binding as previously described.[10,11] Gastrocnemius muscle I-125 α-bungarotoxin binding was used as a measure of acetylcholine receptor number, which increase in response to denervation.[10]

Substance P and its encoding mRNA. Five rats from Group 1 were used for this assay 2 weeks after implantation, approximately 1 week after recovery from neural block deficits. Three ganglia were excised from each cervical (C3–C5) and lumbar (L4–L6) region, bilaterally, immediately frozen on dry ice in four microfuge tubes per rat and homogenized in a 3 M lithium chloride/5 M urea solution. The spun-down pellets were purified for RNA analysis[12] and the supernatants were desalted on C-18 columns for peptide radioimmunoassay. In the radioimmunoassay, unlabeled substance P was competed against Bolten-Hunter ^{125}I labeled substance P with a polyclonal antibody specific for substance P in duplicate samples.[13] The assay was sensitive to 5–10 fM/assay tube. Protein levels eluted with substance P were analyzed with a microtiter plate bicinchoninic protein assay (Pierce, Rockford, IL).

Northern blot analysis of dorsal root ganglia, able to accurately detect 20% differences in RNA levels in single dorsal root ganglia, was developed and described previously.[12] Purified total RNA samples were quantitated with an ethidium bromide Tris-acetate/EDTA gel and equal amounts loaded onto a formaldehyde denatured Northern gel. Relative quantities of messenger RNA encoding for the neuropeptide substance P were normalized to 28S ribosomal RNA (Γ-preprotachykinin/28S rRNA autoradiography grayscale density). Ethidium bromide photonegatives and hybridization autoradiograms were digitized with a flatbed optical scanner and the resulting image analyzed for grayscale density of the signal bands.[12]

The Northern analysis used a full length cDNA of Γ-preprotachykinin,|| previously subcloned into a Promega pGEM-3Z riboprobe vector (Madison, WI) in our laboratory. ^{32}P-UTP labeled riboprobe (specific activity

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|| Γ-preprotachykinin was provided by Dr. J. Krause, Washington University, St. Louis, Missouri.

~10^9 cpm/μg) was made using RNA T7-polymerase (Promega Riboprobe II kit) and purified through a Nick gel permeation size exclusion column (Pharmacia LKB, Piscataway, NJ). A 30-mer oligonucleotide sequence, complementary to a region of rat 28S ribosomal RNA (5'-AAUCCUGCUC AGUACGAGAG GAACCGCAGG-3'), was used for normalization of total RNA loaded into the electrophoretic gel. Twenty nanograms oligonucleotide was ^{32}P end-labeled with the given procedure using T4 polynucleotide kinase (GIBCO BRL, Gaithersburg, MD) and purified on a Nick gel permeation size exclusion column. The specific activity of the probe was greatly reduced (to ~10^5 cpm/μg) by adding 4 μg unlabeled oligonucleotide to the column eluent (400 μl) to reduce the hybridization signal and improve hybridization kinetics.

Statistics

Data were analyzed using linear regression tests (HPLC standards), analysis of variance (sensory block and biochemical data), chi-square tests (motor block data) and Wilcoxon rank-sum tests (biochemical data).

Results

In Vitro *Release*

High-performance liquid chromatography results showed that 96% of the 20 mg bupivacaine incorporated into a 100-mg PLAM pellet (Group 2) was released within 8 days. Because release rate decreased with time, cumulative release increased toward an asymptote. The cumulative release profile was similar for 12% bupivacaine pellets in 10 ml buffer (fig. 2). Group 1 pellets (50 mg) were found to release approximately 75% of the loaded bupivacaine within 4 days *in vitro*. Small differences in release rates were found; lowering the buffer volume slowed bupivacaine release.

In Vivo *Neural Block Measurements*

Group 1 implants (295 ± 10 mg total PLAM in six pellets) in seven animals produced sciatic nerve blockade for periods lasting 2–3 days (fig. 3A). Dense motor blockade was evident in most animals for 2 days. Sensory blockade, measured as increased leg-withdrawal latency to heat in comparison to contralateral control leg, was >200% for day 1 and >70% and >40% for days 2 and 3, respectively. Group 2 implants (295 ± 10 mg total PLAM in three pellets) in six animals
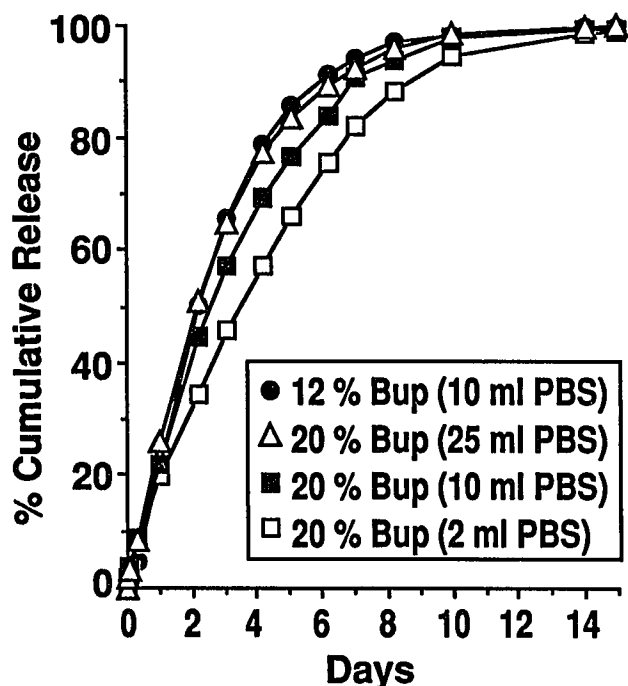
Fig. 2. *In vitro* polymer local anesthetic matrix pellet release studies carried out in various concentrations of phosphate-buffered saline (PBS). Group 2 size pellets were used (see methods). Bup = bupivacaine.

produced sciatic nerve blockade for at least 4 days. Dense motor blockade was observed for 3 days in four animals and 4 days in three animals. Sensory blockade increased leg-withdrawal latency >200% for day 1, >100% for each of days 2 and 3, and >40% for day 4 (fig. 3B). One of the seven rats receiving Group 2 implants did not recover from the surgical implantation procedure. The animal appeared sluggish and lost weight and was therefore eliminated from the study. Group 3 implants (375 ± 10 mg total PLAM in three pellets) in six animals produced partial or complete motor blockade for 4 days and sensory blockade for 4–5 days, including leg-withdrawal latencies that increased more than 185% for the first 3 days, >100% for day 4 and >30% for day 5 (fig. 3C). No impairments were observed on the contralateral control side, implanted with an equal mass of polymer pellets without drug.

Statistical evaluation showed that the hot plate latency data using five measurements per leg was normally distributed in blocked and nonblocked legs (<5% skewness from mean). The mean scores from five measurements per leg were almost identical to the mean scores

found after eliminating the high and low latencies, indicating that the elimination of high and low latencies from the data set to guard against experimental artifact did not produce bias.
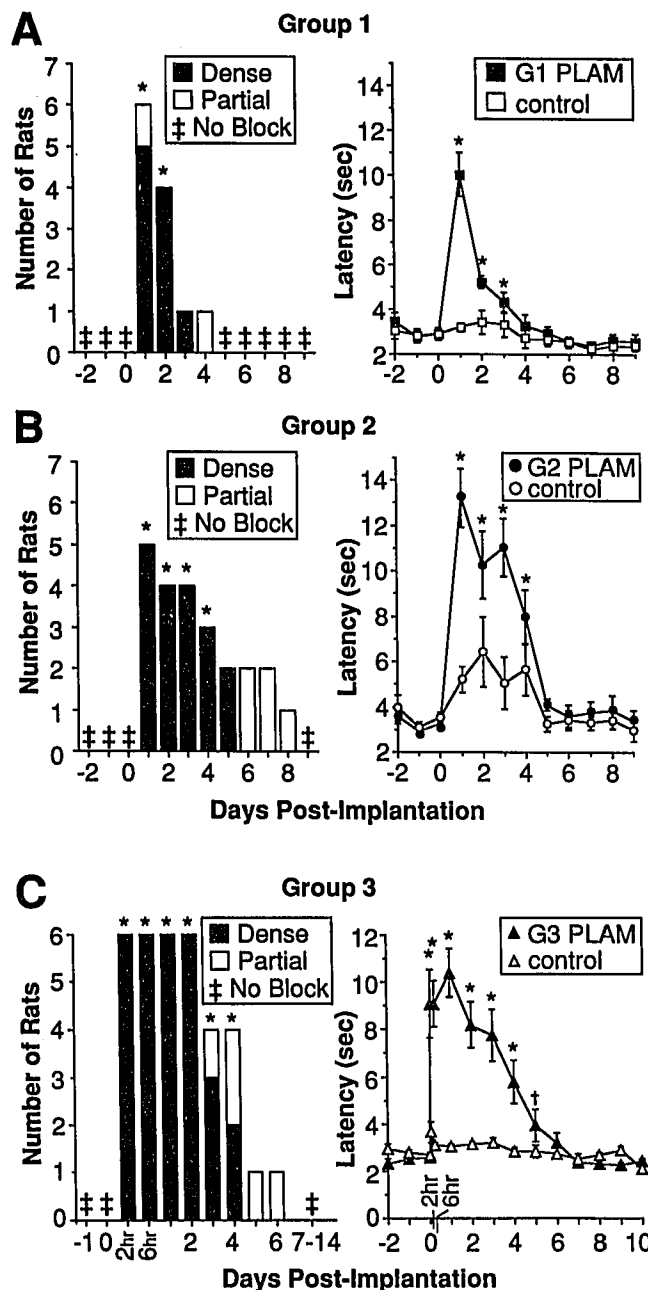

Fig. 3. Nerve block assays. The data for latency represent mean ± SEM. *P < 0.05 significance in comparison to contralateral control side latency (†P = 0.07). In A and B, days 9–14 showed no motor block. (A) Group 1 (n = 7), (B) Group 2 (n = 6), and (C) Group 3 (n = 6).

Table 1. Biochemistry Results of Animals with PLAM Implants: Bupivacaine-treated Leg *versus* Contralateral Control Leg

Analysis	Bupivacaine-treated	Control
Acetylcholine receptor in gastrocnemius muscle (femtomole/mg protein)	44.6 ± 4.1	43.3 ± 2.9*
Substance P content in DRG (femtomole/mg protein)		
Lumbar (n = 7)	0.12 ± 0.01	0.11 ± 0.01*
Cervical (n = 7)	0.08 ± 0.01	0.07 ± 0.01*
Substance P mRNA in DRG (PPT/28S rRNA)		
Lumbar (n = 5)	1.04 ± 0.09	1.03 ± 0.05*
Cervical (n = 4)	0.77 ± 0.10	0.87 ± 0.21*

Values are mean ± SEM.

* $P > 0.3$, bupivacaine-treated *versus* control.

Histology

Sciatic nerve histologic examination showed minimal perineural inflammation with a foreign body response consistent with a local response to previous surgery. Using light microscopy, no evidence of axonal degeneration or demyelination was noted at or proximal or distal to the implantation site.

Biochemical Assays

Prolonged release of local anesthetic and polymer degradation near the sciatic nerve did not lead to differences in any of several biochemical comparisons

made between the side that received PLAM implants and the contralateral control side 2 weeks after implantation (table 1). There was no significant difference found in tests for: (1) acetylcholine receptor number in gastrocnemius muscle[10,11]; (2) the level of substance P, a neuromodulator involved in nociception, in lumbar or cervical dorsal root ganglia[11]; or (3) the level of RNA encoding for substance P, preprotachykinin, in lumbar dorsal root ganglia, using a novel small-sample Northern blot system[11,12] (fig. 4).
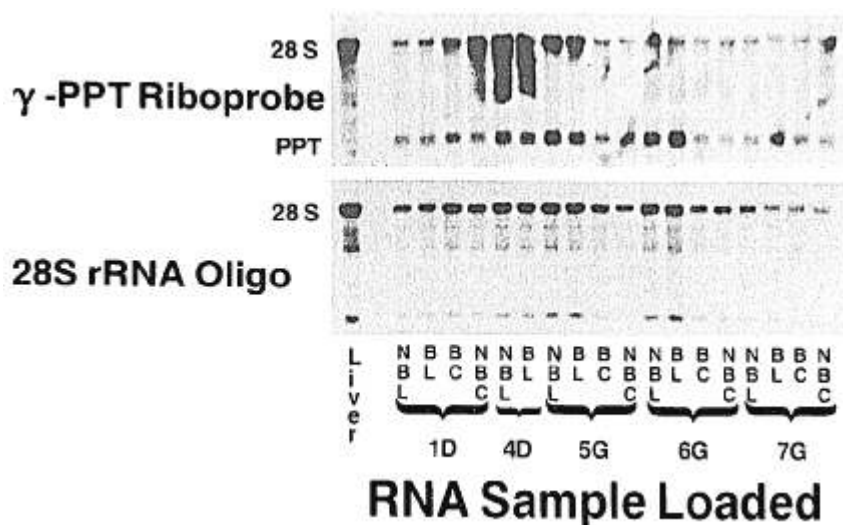
Plasma Bupivacaine Levels

All bupivacaine concentrations in plasma samples collected during the first 4 days after implantation were less than 0.1 μg/ml, below the accurate level of HPLC detectability in plasma samples.

Discussion

Prolonged reversible blockade of the rat sciatic nerve was achieved for periods of 2–6 days *in vivo* using release of bupivacaine from a bioerodible polymer matrix. The results indicate that the increased mass of the PLAM implant increases the period of blockade, suggesting a dose-response relationship. The implants were well tolerated by the animals and produced mild inflammation consistent with the presence of a foreign body. Recovery of motor and sensory function appeared complete. Biochemical results from acetylcholine receptor,[10] substance P,[14] and preprotachykinin[15] assays, showing no differences between PLAM leg and control

Fig. 4. Image of autoradiograms from Northern blot analyses for five rats receiving polymer local anesthetic matrix implants. Each Northern blot autoradiogram was digitized with an optical scanner for display and quantification (results are presented in table 1). Radiolabeled probes were used to measure mRNA levels encoding substance P (preprotachykinin) extracted from dorsal root ganglia tissue (three ganglions, L4–L6) corresponding to the sciatic nerves. The mean grayscale density of autoradiogram signal bands was determined for each specific RNA-probe hybridization.[12] Preprotachykinin (PPT) mRNA levels were normalized to 28S rRNA levels as a measure of total RNA loaded[12] (see methods). Cervical dorsal root ganglia tissue (C3–C5) was used as an additional nonoperated control. N = nonoperated; B = bupivacaine; L = lumbar; C = cervical.

leg, are consistent with a lack of nerve damage. Further studies using electron microscopy are in progress to assess histologic effects of prolonged blockade using these matrixes.

A potential risk of prolonged nerve blockade is systemic accumulation of local anesthetics, leading to convulsion, arrhythmia, and myocardial depression.[16] Plasma concentrations of bupivacaine measured in five rats implanted with Group 1 PLAM pellets (total 60 mg bupivacaine) were below 0.1 μg/ml at 1, 4, 24, 48, 72, and 96 h after implantation,[11] far below the threshold for toxicity of 3–5 μg/ml.[17]

One potential problem with using polymer implants is the formation of fibrous capsules around the PLAM pellets. This encapsulation may prematurely slow the release of drug below an anesthetic blocking dose and block the drug from reaching the nerve. Studies are in progress to inhibit the inflammatory response and collagen production that is associated with encapsulation of the implants. Preliminary results suggest that encapsulation of the polymer implants can be reduced, increasing the duration of blockade (data not shown). It is possible that different degrees of encapsulation along with subtle differences in polymer placement may have contributed to the interanimal variability of observed block.

Implantation of PLAM matrixes could be accomplished in clinical practice either through a surgical field or *via* needles. Potential applications include 2–5-day intercostal blockade for thoracotomy, longer term intercostal blockade for thoracic postherpetic neuralgia, lumbar sympathetic blockade for reflex sympathetic dystrophy, or 3-day ilioinguinal/iliohypogastric blockade for hernia repair. This delivery system can be applied for novel local anesthetic drugs that produce sensory rather than motor blockade[18,19] or that possess physicochemical attributes that make them more useful for sustained release than for single injection blockade.

Long-term application of local anesthetics to peripheral nerves can provide analgesia that would obviate the side effects of systemic opioids. Since polyanhydride polymers are used clinically for sustained release of medications,[4] including intracranial delivery of chemotherapy in patients with brain tumors,[5] PLAM technology may become a useful therapeutic tool.

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