Published in final edited form as:

Biomaterials. 2014 May ; 35(15): 4557–4564. doi:10.1016/j.biomaterials.2014.02.015.

Multivesicular liposomal bupivacaine at the sciatic nerve

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Abstract

Clinical translation of sustained release formulations for local anesthetics has been limited by adverse tissue reaction. Exparel™ (DepoFoam bupivacaine) is a new liposomal local anesthetic formulation whose biocompatibility near nerve tissue is not well characterized. Exparel™ injection caused sciatic nerve blockade in rats lasting 240 minutes compared to 120 minutes for 0.5% (w/v) bupivacaine HCl and 210 minutes for 1.31% (w/v) bupivacaine HCl (same bupivacaine content as Exparel™). On histologic sections four days after injection, median inflammation scores in the Exparel™ group (2.5 of 4) were slightly higher than in groups treated with bupivacaine solutions (score 2). Myotoxicity scores in the Exparel™ group (2.5 of 6) were similar to in the 0.5% (w/v) bupivacaine HCl group (3), but significantly less than in the 1.31% (w/v) bupivacaine HCl group (5). After two weeks, inflammation from Exparel™ (score 2 of 6) was greater than from 0.5% (w/v) bupivacaine HCl (1) and similar to that from 1.31% (w/v)

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bupivacaine HCl (1). Myotoxicity in all three groups was not statistically significantly different. No neurotoxicity was detected in any group. Tissue reaction to Exparel™ was similar to that of 0.5% (w/v) bupivacaine HCl. Surveillance for local tissue injury will be important during future clinical evaluation.

Keywords

Exparel™; DepoFoam; liposome; bupivacaine; inflammation; myotoxicity; neurotoxicity

INTRODUCTION

A wide variety of controlled release local anesthetic formulations has been developed to provide prolonged duration local analgesia (PDLA) from a single injection, including polymeric microspheres[1–6], surgically implantable pellets[7], microcrystals[8], liposomes[9–18], lipospheres[19], cross-linkable hyaluronic acid matrices[20], lipid-protein-sugar particles[21–23], implantable membrane matrices[24, 25], and thermosensitive nanogels[26]. Controlled release PDLA formulations have been used in humans for brachial plexus blockade[27], epidural injection[28, 29], subcutaneous infiltration[30–32], and intercostal nerve blockade[33]. Despite successfully extending the duration of nerve block, they have not been adopted widely. A major limitation to broader clinical use has been adverse tissue reaction, which has included myotoxicity, inflammation, and neurotoxicity, although these have been variably documented in the literature[34]. In animal models, all three are well-recognized sequelae of amino-amide and amino-ester local anesthetics, even after single injections[35–38]. Continuous bupivacaine catheter infusions, even for as brief as 6 hours, result in irreversible skeletal muscle damage in pigs[39]. Controlled release systems themselves enhance tissue injury[4, 20, 22, 40, 41], even when the delivery system is itself minimally toxic (and the same is true in the case of continuous infusions by catheter) [39, 42, 43]. The presence of particles alone causes inflammation at the nerve that considerably outlasts the duration of blockade[4, 6, 20, 22]. Furthermore, local anesthetics are also neurotoxic[44–46]. In our hands, tissue injury has been a feature of all PDLA formulations containing amino-amide local anesthetics we have tested, and injury was most directly attributable to the drug, not the vehicle[4, 6, 20, 22, 40, 41].

The risk of clinically significant severe local tissue reaction in humans is more than a theoretical concern[47–49]. Persistent diplopia from extraocular muscle injury can occasionally occur following a single injection of local anesthetic[49]. That tissue injury is a crucial consideration for PDLA systems is seen in the example of a sustained-release bupivacaine-dexamethasone formulation where inflammation and nerve and muscle injury in preclinical animal studies and clinical human trials led to withdrawal of its Investigational New Drug application (IND#53,441)[34]. Despite evidence suggesting that tissue injury is an important issue for all PDLA formulations containing amino-amide (and presumably amino-ester) local anesthetics, it is often not documented[1–3, 8–10, 50] (or even reported to be absent)[5]. When recognized, tissue injury is generally observed as mild granulomatous inflammation[7, 11, 13, 14, 19]. Muscle injury is rarely described and when it is, it is

characterized as mild (or no different than saline injection), self-limited and similar to single injection of free drug[24, 25].

Translation of PDLA technologies into the clinic has been slow. A liposomal bupivacaine formulation (DepoFoam bupivacaine, Exparel™) has now become commercially available and has been used in multiple human trials of infiltration local anesthesia in various soft tissues [12, 15–18, 51, 52]. Pre-clinical animal studies had been generally reassuring: mild granulomatous inflammation had been reported to be the major histopathologic consequence[11, 13, 14]. Myotoxicity has been reported as being mild [11] or absent [13] or not commented upon [14]. Neurotoxicity was not seen in an animal study of brachial plexus blockade, but the histological toll was hematoxylin-eosin staining [13], which is insensitive for detecting nerve injury.

Recently, Exparel™ was used for femoral nerve blockade in healthy human volunteers and produced partial sensory and motor blockade for up to 24 hours[53]. This study was not preceded by adequate published studies of Exparel™'s biocompatibility in proximity to major nerves and muscles, an environment in which severe injury is potentially possible, and in which caution has been suggested in the use of some PDLA formulations[54]. Here, we used an established rat model[4, 6, 20, 22, 40, 41, 55–60] to determine the efficacy of Exparel™ in sciatic nerve blockade and to describe the resulting local tissue injury (inflammation, myotoxicity, and neurotoxicity). We compare Exparel™ to 0.5% (w/v) bupivacaine hydrochloride (HCl) (a concentration of bupivacaine in clinical practice) and 1.31% (w/v) bupivacaine HCl (a concentration of bupivacaine equal to that in Exparel™ but too high to be used clinically).

MATERIALS AND METHODS

Materials

Exparel™ (multivesicular liposomal bupivacaine suspended in 0.9% sodium chloride; 13.3 mg bupivacaine/mL) was purchased from Pacira Pharmaceuticals, Inc. (San Diego, CA). Bupivacaine Hydrochloride was purchased from Sigma-Aldrich, Co. (St. Louis, MO).

Isolation of liposomes from Exparel™ suspension and characterization

The contents of a 20 mL vial were centrifuged at 6000 rpm for 20 minutes and the supernatant decanted. The liposomal pellet was lyophilized for 24 hours and then suspended in phosphate buffered saline (PBS) ($10 \text{ mg} \cdot \text{mL}^{-1}$) and sonicated (Fibrocell VCX 500, 3 mm stepped microtip, Sonics and Materials, Inc., Newtown, CT) at 100 Watts with a 1 second alternating on/off cycle for 20 seconds to destroy the liposomes. After additional dilution in PBS the bupivacaine concentration was measured in the supernatant or sonicated liposomal suspension by high-performance liquid chromatography (Agilent 1200 series, Agilent Technologies, Inc., Wilmington, DE) with a 150 mm, $2.7 \mu\text{m}$ particle Poroshell 120 column (Agilent Technologies, Inc., Wilmington, DE). The mobile phase consisted of 0.05% (v/v) trifluoroacetic acid in 1:1 acetonitrile and water with a flow rate of 1 mL/min.

Liposome size was determined with a Coulter Multisizer (Coulter Electronics Ltd., Luton, United Kingdom).

***In Vitro* Release of Bupivacaine from liposomes**

One mL of Exparel™ was placed into the lumen of a Spectra/Por 1.1 dialysis bag with an 8–10 kD molecular weight cutoff (Spectrum Laboratories, Ranch Dominguez, CA). The dialysis bag was placed into 20 mL phosphate-buffered saline, pH 7.4, and incubated at 37 °C with continuous stirring. At predetermined time intervals, the dialysis bag was transferred to fresh PBS. The bupivacaine concentration in the dialysate was quantified by comparing the absorbance at 272 nm to a standard curve.

Animal Care

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 310–420 g were housed in groups, in a 6 AM–6 PM light-dark cycle. Animals were cared for in accordance with protocols approved by the Animal Care and Use Committee at the Massachusetts Institute of Technology (Cambridge, Massachusetts), and the *Guide for the Care and Use of Laboratory Animals* of the US National Research Council.

Sciatic Blockade Technique

Nerve block injections were performed with a 20-gauge needle under isoflurane-oxygen anesthesia as described[55, 57]. Rats were injected with 0.6 mL of Exparel™ (approximately 25 mg · kg⁻¹ total dose of bupivacaine per injection), 0.5% (w/v) bupivacaine HCl (approximately 10 mg · kg⁻¹ total dose of bupivacaine per injection) or 1.31% (w/v) bupivacaine HCl (approximately 25 mg · kg⁻¹ total dose of bupivacaine per injection). The needle was introduced posteromedial to the greater trochanter pointing in an anteromedial direction. Once bone was contacted, the liposomal suspension was injected.

Assessment of Nerve Blockade

The effectiveness of block was measured at predetermined time points using methods previously described[7, 55, 57]. Hind paws were exposed in sequence (left, then right) to a 56°C hot plate (model 39D Hot Plate Analgesia meter; IITC Inc., Woodland Hills, CA), and the time until paw withdrawal (thermal latency) was measured. The data are reported in terms of thermal latency (intensity) and duration of block. Thermal latency is a measure of the degree of analgesia. If the animal did not remove its paw within 12 seconds, it was removed to avoid injury or the development of hyperalgesia. Latency was measured in the uninjected leg and used as a control for systemic effects. The duration of thermal nociceptive block was calculated as the time required for thermal latency to return to a value of 7 seconds from a higher value. Seven seconds is the midpoint between maximal block and normal thermal latency (approximately 2 seconds) in adult rats, and a maximal latency of 12 seconds[57].

Tissue Harvesting and Histology

After euthanasia with carbon dioxide the sciatic nerve and surrounding muscle were harvested. Muscle specimens were processed to produce hematoxylin and eosin-stained slides. A gross dissection score was given as follows: 0 = tissue planes obvious and easily separated; 1 = tissue planes obvious but separated with some difficulty; 2 = tissue planes adherent and separated with some difficulty; 3 = tissue planes completely obliterated, could

not separate surrounding tissues from nerve without cutting through them[22]. Muscle samples were scored for inflammation (0–4) and myotoxicity (0–6)[60]. The inflammation score was a subjective assessment of severity. The myotoxicity score reflected two characteristic features of local anesthetic myotoxicity: nuclear internalization and regeneration. Nuclear internalization is characterized by myocytes normal in size and chromicity, but with nuclei located away from their usual location at the periphery of the cell[4]. Regeneration is characterized by shrunken myocytes with basophilic cytoplasm[39]. Scoring was as follows: 0 = normal; 1 = perifascicular internalization; 2 = deep internalization (>5 cell layers), 3 = perifascicular regeneration, 4 = deep regeneration, 5 = hemifascicular regeneration, 6 = holofascicular regeneration.

For histology of nerve injury, samples were processed for Epon-embedding and fixed in Karnovsky's KII Solution (2.5 % glutaraldehyde, 2.0 % paraformaldehyde, 0.025 % calcium chloride in 0.1M sodium cacodylate buffer, pH 7.4). Samples were post-fixed in osmium tetroxide, stained with uranyl acetate, dehydrated in graded ethanol solutions, and infiltrated with propylene oxide/Epon mixtures. 0.5 μm sections were stained with toluidine blue for high-resolution light microscopy. Neuropathologic changes were minimal for all injection groups. Therefore quantitative analysis of neurotoxicity was not performed.

Statistical Analysis

Data are reported as medians with 25th and 75th percentiles, and are compared using the unpaired Mann-Whitney U test. This method was selected because the data were ordinal (dissection, inflammation and myotoxicity scores), or because they were not normally distributed (neurobehavioral data). To avoid type I error in comparisons between gross dissection, inflammation and myotoxicity scores, and nerve block durations, we used Bonferroni corrections, where the P-value (α) required for statistical significance was determined by dividing 0.05 by the number of comparisons. Two planned comparisons were performed between groups. Therefore, $\alpha = 0.05/2 = 0.025$, so a P -value < 0.025 was required for statistical significance.

RESULTS

Particle characteristics

ExparelTM liposomes appeared spherical by phase contrast microscopy (Figure 1). Mean particle diameter was $31.2 \mu\text{m} \pm 17.8$ ($n = 4$). They were separated by centrifugation and the supernatant and isolated liposomes were analyzed for bupivacaine content. Centrifugation of a 20 mL vial at 6000 rpm yielded 550.27 mg of liposomal formulation and 12.5 mL of supernatant. The measured bupivacaine content of the liposomal fraction was 260.52 ± 18.33 mg ($n=4$, cf. 266 mg based on the bupivacaine concentration of $13.3 \text{ mg} \cdot \text{mL}^{-1}$ (1.33% [w/v]) reported by the manufacturer); the percent bupivacaine loading was therefore 47.37 ± 3.33 % (w/w, wet). The bupivacaine concentration in the supernatant was $0.10 \pm 0.01 \text{ mg} \cdot \text{mL}^{-1}$ (0.010% [w/v]), i.e. a total of 1.25 ± 0.07 mg of bupivacaine in 12.5 mL of supernatant ($n=4$). Thus the total measured bupivacaine content of ExparelTM was $13.05 \pm 0.91 \text{ mg} \cdot \text{mL}^{-1}$, or 261.77 ± 18.28 mg in each 20 mL vial.

Drug release from liposomes

Dialysis bags with an 8–10 kDa molecular weight cutoff were filled with 1 mL of Exparel™ (13.05 mg of bupivacaine) as purchased from the manufacturer, then placed into 20 mL phosphate buffered saline at pH 7.4 and 37 °C. One mL of 0.5% (w/v) or 1.31% (w/v) unencapsulated bupivacaine HCl (containing the same amount of bupivacaine as 1 mL of Exparel™) was treated in the same way. Release of bupivacaine into the dialysate was measured spectrophotometrically and expressed as cumulative release (Figure 2). Release of bupivacaine from Exparel™ was slowed compared to that of unencapsulated drug. By 48 h $99.0 \pm 0.9\%$ ($n = 4$) of free bupivacaine had been released from the dialysis bags containing 1.31% (w/v) bupivacaine HCl, compared to $9.7 \pm 4.1\%$ of bupivacaine from bags containing Exparel™ ($p < 0.001$). The release kinetics of 0.5% (w/v) bupivacaine HCl were similar to that of 1.31% (w/v) bupivacaine HCl. Release of bupivacaine from Exparel™ was complete by approximately 800 h of release.

Duration of sciatic nerve blockade

Animals received injections with 0.6 mL of Exparel™ as purchased, 0.5% (w/v) bupivacaine hydrochloride or 1.31% (w/v) bupivacaine hydrochloride at the sciatic nerve ($n=10$ for all groups). Maximal sensory blockade in our testing paradigm (latency = 12 s) was achieved in all animals for each group. The median duration of sensory blockade (Table 1) resulting from Exparel™ (240 minutes) was twice as long as that from 0.5% (w/v) bupivacaine HCl (120 minutes), but only slightly longer than 1.31% (w/v) bupivacaine HCl (210 minutes).

Systemic distribution of bupivacaine

We used the presence of functional deficits in the contralateral (uninjected) extremity as a measure of the degree of systemically distributed bupivacaine. Functional deficits were quantified by comparing peak median thermal latency in the contralateral hind limb to baseline thermal latency in the same limb. Sensory deficits were not detected in the contralateral hind limbs of animals injected with Exparel™ ($p=1.00$) or 0.5% (w/v) bupivacaine HCl ($p=0.31$). However, injection with 1.31% (w/v) bupivacaine HCl prolonged contralateral thermal latency from 2.7 (2.3–3.1) to 4.4 (3.4–4.8) seconds ($p=0.005$).

Tissue Reaction

The sciatic nerves with surrounding tissues were removed 4 days or 14 days after injection and processed for histology ($n=6$ for all groups). All rats injected with Exparel™ had white, gelatinous deposits of particle residue in discrete pockets directly adjacent to the sciatic nerve at 4 and 14 days (Figure 3). The particle residue appeared subjectively much smaller at two weeks after injection. On histologic examination there was evidence of some degree of inflammation and myotoxicity in all animals (Figure 4).

Tissue reaction was evaluated at 4 days after injection, when acute inflammation and muscle injury is often well established after perineural injection of a drug delivery system[22] (Table 2, $n=6$). The gross appearance for animals injected with Exparel™ or 0.5% (w/v) bupivacaine HCl was notable for tissue planes at the injection site that were mildly adherent

to each other, i.e. separated with minimal effort (Figure 3). Tissues injected with 1.31% (w/v) bupivacaine HCl were hyperemic and tissue planes were adherent (this was not seen with Exparel™ or 0.5% (w/v) bupivacaine HCl). The median dissection score—a measure of the difficulty of dissection that is affected by inflammation, with a range of 0 to 3—in animals injected with Exparel™ was the same as in animals injected with 0.5% (w/v) bupivacaine HCl (1.0), but less than in animals injected with 1.31% (w/v) bupivacaine HCl (2.0; $p = 0.018$). On histologic examination, the tissue reaction to Exparel™ consisted of a mixed inflammatory infiltrate consisting primarily of macrophages admixed with a smaller population of lymphocytes and occasional neutrophils in the soft tissues surrounding the muscle (Figure 4a,d). The macrophages had foamy cytoplasm, likely reflecting uptake of the lipid-rich Exparel™ particles; these foamy macrophages were not seen in the 0.5% (w/v) or 1.31% (w/v) bupivacaine formulations. There was evidence of myotoxicity from the Exparel™ reflected by degenerating and regenerating myocytes predominantly in the perifascicular region of the muscle bundle. The tissue reaction to the 0.5% (w/v) bupivacaine was similar in the extent of inflammation and myotoxicity, but lacked the foamy macrophage population (Figure 4b,e). In contrast, the tissue reaction to the 1.31% (w/v) bupivacaine demonstrated significant myotoxicity manifest as frank coagulation necrosis of the myocytes in the perifascicular region, with degenerating and regenerating myocytes occupying approximately half of the muscle bundle on average (Figure 4c,f). The median inflammation scores (range: 0–4) in animals injected with Exparel™ (2.5), 0.5% (w/v) bupivacaine HCl (2.0) and 1.31% (w/v) bupivacaine HCl (2.0) were similar (Table 2). The median myotoxicity score (range: 0–6) in animals injected with Exparel™ (2.5) was similar to that in animals injected with 0.5% (w/v) bupivacaine HCl (3.0) but less than that in animals injected with 1.31% (w/v) bupivacaine HCl (5.0; $p=0.011$).

Tissue reaction was examined at 14 days (Table 2, $n=6$), when inflammation has become chronic[61] and/or may be resolving[22] and muscle injury is recovering from short-acting formulations[22]. In animals injected with Exparel™ and 0.5% (w/v) bupivacaine HCl, gross tissue appearance at two weeks was similar to that at 4 days (Figure 3). The adherence of tissues to each other seen in the 1.31% (w/v) bupivacaine HCl group at 4 days had resolved by 14 days (Figure 3). The median dissection score at 14 days was higher in animals injected with Exparel™ (2.0) than in animals injected with 0.5% (w/v) bupivacaine HCl (0.0; $p = 0.022$) but not 1.31% (w/v) bupivacaine HCl (1.5). On histologic examination, the tissue reaction to Exparel™ was diminished compared to the 4 day time point, but qualitatively similar with foamy macrophages and lymphocytes. There was diminished myotoxicity as well, with mild changes limited largely to the perifascicular region of the muscle bundle (Figure 4h,k). There was less inflammation (and no foamy macrophages) associated with the 0.5% (w/v) bupivacaine, but the degree of myotoxicity was similar to that of the Exparel™ (Figure 4i,l). The histologic findings in the 1.31% (w/v) bupivacaine group consisted of inflammation and regenerative changes that were diminished relative to the 4 day time point, but were more severe than seen in the other groups (Figure 4j,m). The median inflammation score for Exparel™ (2.0) was statistically significantly higher than for 0.5% (w/v) bupivacaine HCl (1.0; $p=0.006$) but not than for 1.31% (w/v) bupivacaine HCl (1.0). The median myotoxicity scores for Exparel™ (1.0) was similar to that for 0.5% (w/v)

bupivacaine HCl (1.5) and for 1.31% (w/v) bupivacaine HCl (3.0; although $p=0.029$, that difference was not statistically significant after Bonferroni correction).

At 4 and 14 days, toluidine blue stained sciatic nerve samples were harvested from rats administered Exparel™, 0.5% (w/w) bupivacaine HCl or 1.33% (w/v) bupivacaine HCl. Findings were similar for all treatments, regardless of time point. No significant changes in axonal density or myelin structure were observed. The perineural tissue appeared normal. Figure 4 shows representative images at 4 days. Appearance at 14 days was similar and is not shown.

DISCUSSION

The principal question addressed in this work was to what extent the liposomal bupivacaine formulation Exparel™ (DepoFoam bupivacaine) causes local tissue injury, particularly myotoxicity and neurotoxicity, when used in an animal model of regional (major nerve) blockade. The importance of this matter has been highlighted by the recent study of this formulation in humans for regional anesthesia[53], in the absence of any such animal data.

Exparel™ caused similar degrees of myotoxicity to 0.5% (w/v) bupivacaine HCl at 4 days and 2 weeks after injection (Table 2) in an established rat model[4, 6, 20, 22, 40, 41, 55, 60]. Exparel™ was less myotoxic and appeared to result in less systemic drug distribution than did an equal concentration of un-encapsulated bupivacaine HCl (1.31% (w/v)). These beneficial effects were presumably due to control of the release of bupivacaine [4, 62]

Nonetheless, it may be premature to dismiss the risk of untoward tissue reaction to Exparel™ or any other sustained release local anesthetic, for the following reasons. First, the mechanisms by which sustained release of local anesthetics cause severe tissue injury is incompletely understood, although various aspects of drug release kinetics have been implicated, as has the presence of a delivery vehicle [4]. The potential for significant tissue injury might be further increased when a PDLA formulation is used in anatomic locations with relatively low blood flow, or in patients with poor peripheral circulation, as these conditions might lead to the maintenance of high local tissue concentrations. Second, at this time, it is difficult to know what degrees of adverse tissue reaction in animal studies would translate into adverse events in human subjects when such formulations are used near nerves and major muscles. Third, clinically significant muscle injury from local anesthetic myotoxicity is rare but well documented and can cause significant morbidity[47–49]. In a clinical trial of intercostal nerve block with polymeric microspheres containing bupivacaine and dexamethasone, 1 in 36 patients developed prolonged (3 months) paresthesia[33]. In the retrospective view of what befell that formulation (withdrawal of the investigational new drug [IND] because of tissue injury in animals and humans[34]), that paresthesia was possibly a forme fruste of nerve injury.

We have observed inflammation and myotoxicity with a broad range of delivery systems with very different compositions of matter, including PLGA[4, 6, 22], lipid-sugar-protein particles[22, 57], polysaccharide-based gels[20, 63], and thermosensitive nanogels[64], suggesting the possibility that some degree of muscle injury is an unavoidable concomitant

of all PDLA formulations that use conventional local anesthetics, including Exparel™. In some cases, such as with cross-linked hyaluronic acid hydrogels containing bupivacaine, tissue reaction was comparable to that from the drug alone [20]. With Exparel™, tissue reaction was more benign than that of the equivalent concentration of the unencapsulated drug (1.31% (w/v) bupivacaine HCl; a toxic concentration that would not be used clinically), and comparable to that of a common clinical concentration (0.5% (w/v) bupivacaine HCl). With the cross-linked hyaluronic acid, the duration of nerve block was increased by a relatively modest factor of two compared to the free drug [20], and with Exparel™ the duration of block was only increased by approximately 14% compared to 1.31% (w/v) bupivacaine HCl. It is not known whether the relatively mild tissue reaction from those formulations could occur with systems that provided longer nerve blockade using the same drugs. It bears mentioning in this context that we and others have found that site 1 sodium channel blockers such as tetrodotoxin and saxitoxin cause minimal to no tissue injury (specifically, no myo- or neurotoxicity) when used as local anesthetics[44, 60, 65]. Site 1 sodium channel blockers can produce prolonged duration local anesthesia lasting many hours to days depending on the formulation[44, 59, 66, 67].”

The duration of sensory block achieved by Exparel™, was approximately twice that achieved with a commonly used concentration of bupivacaine HCl (0.5% [w/v]). It remains to be seen whether that degree of prolongation finds a niche in clinical practice. The preliminary experience with regional nerve blocks with Exparel™ in humans is that it produced partial motor and sensory blockade for approximately 24 hours; no adverse reactions were reported[53]. The discrepancy between the durations of block in animals and humans may have had to do with differences in the testing paradigms (e.g. testing for different intensities of analgesia), as well as technical differences (different nerve block, differences in scaled volume of injectate etc.).

Local anesthetics, particularly PDLA formulations, are associated with myotoxicity and inflammation. Exparel™ caused more local tissue inflammation two weeks after injection than did either 0.5% (w/v) or 1.31% (w/v) bupivacaine HCl. This long-lasting inflammation was also documented in previous Exparel™ studies[11, 13, 14]. The presence of drug delivery systems themselves has been shown to cause inflammation that outlasts the duration of nerve blockade[4, 6, 20, 22] and may enhance local anesthetic myotoxicity[4, 20]. The potential detrimental effects of persistent inflammation near a nerve are unknown. However, we have previously shown that the presence of inflammation from polymeric microspheres at the sciatic nerve for 4 and 7 days has minimal effects on gene expression in the associated dorsal root ganglia (where the sensory bodies are), and does not cause up-regulation of markers for nerve injury[68].

It could be argued that the tissue reaction to Exparel™ observed here provides reassurance regarding its use near major nerves and muscles. However, given the relatively little that is known regarding the incidence of and contributing factors to tissue toxicity from PDLA, we feel that caution will remain important when using any PDLA formulations based on conventional (amino-amide and amino-ester) local anesthetics near such structures. Surveillance for complications will be crucial. These considerations are perhaps particularly germane to Exparel™ at this time, given the proliferation of clinical trials of that product[12,

15–18, 51, 53, 69], reports that it will soon be tested for epidural and intra-articular use[13], and the possibility of practitioners being tempted by the off-label use of drugs and devices.

CONCLUSION

In summary, Exparel™ caused myotoxicity comparable to that from 0.5% (w/v) bupivacaine HCl, although inflammation lasted much longer. It will be crucial for the clinician to be aware of the risk of tissue injury as agents of this kind are introduced into broader clinical use and for surveillance for tissue injury to be performed diligently.

Acknowledgments

This research was funded by NIH GM 073626 (to DSK). None of the investigators have any financial connection to Exparel™, or Pacira Pharmaceuticals.

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Figure 1.
Phase contrast photomicrograph of Exporel™. Scale bar = 30 μm.

Figure 2.

Cumulative release of bupivacaine from Exparel™. Also shown is the release of unencapsulated 1.31% (w/v) and 0.5% (w/v) bupivacaine HCl. Data are means $\pm$ standard deviations, $n=4$.

Figure 3.

Representative photographs 4 days and 14 days after injection at the sciatic nerve with Exparel™, 0.5% (w/v) bupivacaine HCl or 1.31% (w/v) bupivacaine HCl.

Figure 4.

Representative light microscopy of hematoxylin/eosin-stained sections of adjacent muscle (M) 4 and 14 days after injection at the sciatic nerve with Exparel™, 0.5% (w/v) bupivacaine HCl or 1.31% (w/v) bupivacaine HCl. 4 days after injection with (A and B) Exparel™ or 0.5% (w/v) bupivacaine HCl, myotoxicity (Mtox) and inflammation (Infl) were predominantly perifascicular; for (C) 1.31% (w/v) bupivacaine HCl, only the deepest layers were spared. (D–F) At 4 days myotoxicity and inflammation, regardless of treatment group, were characterized by regenerating myocytes (Regen) surrounded by macrophages and occasional polymorphonuclear cells and lymphocytes. (D) For Exparel™, foamy macrophages (FM) with ingested particulate matter could be seen. 14 days after injection with (H and I) Exparel™ or 0.5% (w/v) bupivacaine HCl, tissue injury

was limited to the periphery of the fascicle and was characterized by (K and L) myocytes with centralized nuclei surrounded by occasional lymphocytes; for (J) 1.31% (w/v) bupivacaine HCl, perifascicular myotoxicity and holofascicular inflammation were observed and was characterized by (M) regenerating myocytes surrounded by vacuolated macrophages and lymphocytes. Scale bars represent 100 μm (A–C, H–J) or 20 μm (D–F, K–M).

Figure 5.

Toluidine blue stained sciatic nerve samples harvested from rats 4 days after injection with either (A) Exparel™, (B) 0.5% (w/v) bupivacaine HCl or (C) 1.31% (w/v) bupivacaine HCl demonstrated normal findings. No significant changes in axonal density or myelin structure were observed. The perineurial tissue appeared normal. (D) Normal, uninjected sciatic nerve. Scale bars represent 100 μm.

Table 1

Duration of sensory nerve blockade (minutes)

Exparel™	240 (240-240)
0.5% (w/v) bupivacaine HCl	120 (120-165)
P value	0.001
1.31% (w/v) bupivacaine HCl	210 (180-240)
P value	0.013

P values result from the comparison of Exparel™ to 0.5% (w/v) or 1.31% (w/v) bupivacaine HCl (Mann-Whitney U test).

Data are median values with 25th and 75th percentiles; n=10 for all groups.

P values < 0.025 are considered statistically significant.

Table 2

Values for the 3 measures of biocompatibility for each treatment group

	Score	
	Day 4	Day 14
Gross dissection (0–3)		
Exparel™	1.0 (1.0–2.0)	2.0 (1.3–2.0)
0.5% (w/v) Bupivacaine HCl	1.0 (0.0–2.0)	0.0 (0.0–0.8)
P value ^a	0.48	0.022
1.31% (w/v) Bupivacaine HCl	2.0 (2.0–2.3)	1.5 (1.0–2.0)
P value ^b	0.018	0.45
Inflammation (0–4)		
Exparel™	2.5 (2.0–3.0)	2.0 (1.8–2.0)
0.5% (w/v) Bupivacaine HCl	2.0 (2.0–2.0)	1.0 (0.8–1.0)
P value ^a	0.056	0.006
1.31% (w/v) Bupivacaine HCl	2.0 (2.0–2.0)	1.0 (1.0–2.0)
P value ^b	0.056	0.093
Myotoxicity (0–6)		
Exparel™	2.5 (1.0–3.3)	1.0 (1.0–2.0)
0.5% (w/v) Bupivacaine HCl	3.0 (3.0–4.0)	1.5 (1.0–2.0)
P value ^a	0.12	0.58
1.31% (w/v) Bupivacaine HCl	5.0 (3.8–5.0)	3.0 (1.8–4.0)
P value ^b	0.011	0.029

P values result from the comparison of Exparel™ to ^a 0.5% (w/v) or ^b 1.31% (w/v) bupivacaine HCl (Mann-Whitney U test).

Data are median values with 25th and 75th percentiles; n=6 for all groups.

P values < 0.25 are considered statistically significant.