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Local Toxicity from Local Anesthetic Polymeric Microparticles

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Abstract

Background—Local tissue injury from sustained release formulations for local anesthetics can be severe. There is considerable variability in reporting of that injury. We investigated the influence of the intrinsic myotoxicity of the encapsulated local anesthetic (lidocaine, low; bupivacaine, high) on tissue reaction in rats.

Methods—Cytotoxicity from a range of lidocaine and bupivacaine concentrations was measured in C2C12 myotubes over 6 days. Rats were given sciatic nerve blocks with 4 microparticulate formulations of lidocaine and bupivacaine: 10% (w/w) lidocaine poly-lactic-co-glycolic acid (PLGA), 10% (w/w) bupivacaine PLGA, 50% (w/w) lidocaine PLGA, and 50% (w/w) bupivacaine PLGA. Effectiveness of nerve blockade was assessed by a modified hotplate test and weight-bearing measurements. Myotoxicity was scored in histologic sections of injection sites. Bupivacaine and lidocaine release kinetics from the particles were measured.

Results—Median sensory blockade duration for 50% (w/w) lidocaine was 255 (90–540) min versus 840 (277–1215) min for 50% (w/w) bupivacaine ($P=0.056$). All microparticulate formulations resulted in myotoxicity. The choice of local anesthetic did not influence the severity of myotoxicity. Median myotoxicity scores for 50% (w/w) lidocaine compared to 50% (w/w) bupivacaine at 4 days was 3.4 (2.1–4.2) vs. 3.3 (2.9–3.5) ($P=0.44$) and at 14 days 1.9 (1.8–2.4) versus 1.7 (1.3–1.9) ($P=0.23$) respectively.

Conclusions—Lidocaine and bupivacaine PLGA microspheres resulted in similar degrees of myotoxicity, irrespective of drug loading. Intrinsic myotoxicity did not predict tissue injury from sustained release of these anesthetics. Caution is warranted in the use of such devices near muscle and nerve.

Introduction

A very broad range of controlled release formulations have been developed to provide prolonged duration local anesthesia, including polymeric microspheres,^{1–7} surgically implantable pellets,⁸ microcrystals,⁹ liposomes,^{10–15} (including a formulation undergoing human clinical trials^{16,17}) lipospheres,¹⁸ cross-linkable hyaluronic acid matrices,¹⁹ lipid-protein-sugar particles,^{20,21} cyclodextrin complexes,^{22,23} liposomes loaded with cyclodextrin complexes²⁴ and implantable membrane matrices.^{25,26} Such systems have extended the duration of nerve block to varying degrees ranging from hours to weeks, but have not been widely adopted clinically. A major limitation has been adverse tissue reaction, which has included inflammation, myotoxicity and neurotoxicity. All three are well-recognized sequelae of amino-amide and amino-ester local anesthetics.^{27–32} The degree of toxicity is agent specific.^{28,30} In a comparison of multiple drugs in rats, single injections of lidocaine produced milder muscle damage than bupivacaine.³⁰ Local anesthetic myotoxicity from single injections has not generated much clinical concern, although the consequences from multiple large doses^{33,34} or from continuous infusions can be severe and are related to the duration of exposure.^{35,36} The presence of particles themselves enhances local anesthetic myotoxicity in vivo⁶ and can cause inflammatory responses at the nerve that may considerably outlast the duration of blockade.^{6,21,37} Conventional local anesthetics are also neurotoxic, but generally at higher concentrations than those that cause myotoxicity and inflammation.^{32,38–40}

Reporting of local tissue injury from local anesthetic controlled release formulations has been variable, in both animal and human studies. Most do not describe myotoxicity^{2-5,7-9,11-15,18,22-24} (including the liposomal formulation undergoing human trials^{16,17}), while some others document mild muscle injury comparable to single injections of unencapsulated drug.^{10,25,26} In our own work, we have found muscle injury to be a ubiquitous finding in a wide range of extended-release bupivacaine formulations independent of the delivery vehicle^{6,19,21,41} or co-encapsulated agent,^{32,37,42,43} and it is sometimes severe. That tissue injury can be a crucial issue with sustained release formulations is seen in the example of a sustained-release bupivacaine-dexamethasone formulation;³ nerve and muscle injury in preclinical animal studies and clinical human trials led to withdrawal of its Investigational New Drug application (IND#53,441).⁴⁴

There are many possible reasons for the variability in reporting of local anesthetic myotoxicity. There is marked heterogeneity in the literature of delivery systems, drug selection, experimental designs, and histopathologic descriptions. Furthermore, the particular drug encapsulated varies; as noted above, that could have an effect on local tissue reaction.

Here we sought to determine whether tissue reaction to a particular formulation is dependent on the specific drug that is encapsulated, by encapsulating a local anesthetic with either high (bupivacaine) or low (lidocaine) potency and myotoxic potential. Such differential toxicity might explain why some lidocaine-releasing formulations have been reported to cause relatively minimal toxicity (comparable to free drug),²⁵ which has not been our experience with bupivacaine-containing formulations. We also examined whether drug loading affects tissue reaction. We selected microspheres composed of high molecular weight poly (lactic-co-glycolic) acid (PLGA), a biodegradable polymer widely used in drug delivery and other applications. Blank PLGA particles produce minimal toxicity in cultured C2C12 myotubes and when injected at the sciatic nerve.⁶

Methods

Materials

Bupivacaine hydrochloride, lidocaine free base, Tween 80 and sodium carboxymethyl cellulose were purchased from Sigma-Aldrich (St. Louis, MO), poly(lactic-co-glycolic) acid (lactide/glycolide = 65:35, MW 110) (PLGA₁₁₀) from Medisorb (Alkermes; Cambridge, MA), and poly(vinyl alcohol) (88% hydrolyzed, MW 20,000) from Polysciences, Inc. (Warrington, PA). Bupivacaine hydrochloride was made into the free base by alkaline precipitation and filtration.

Microparticle preparation and characterization

Microspheres with 10% (w/w) and 50% (w/w) drug loading were prepared using the single emulsion method.^{4,20} Drug free base (50 mg bupivacaine or 175 mg lidocaine for 10% loaded particles; 160 mg bupivacaine or 2 g lidocaine for 50% loaded particles) and PLGA (100 mg for all formulations except 50% lidocaine, which required 600 mg) were dissolved in methylene chloride (3 mL for 50% loaded lidocaine particles; 1.26 mL for all others), and the mixture homogenized (Silverson L4RT-A; Longmeadow, MA) in 50 mL 0.5% polyvinyl alcohol in 100 mM Trizma buffer pH 10.5 for one minute. Lidocaine microparticles required more free base in the starting materials than bupivacaine, owing to the lower octanol:buffer partition coefficient of lidocaine. The resulting suspension was decanted into 100 mL 0.05% polyvinyl alcohol in 100 mM Trizma pH 10.5 and stirred to evaporate the methylene chloride. Microspheres 20 μ m to 106 μ m in diameter were isolated by wet sieving then

resuspended in 50 ml of water. The suspension was washed three times by centrifugation at 4000 rpm for 5 minutes.

Particle size was determined with a Coulter multisizer (Coulter Electronics Ltd., Luton, United Kingdom). Drug loading was determined by dissolving 10 mg of microspheres in 1 mL of methylene chloride, and comparing the resulting UV absorbance (272 nm for bupivacaine, 265 nm for lidocaine) to a standard curve. Blank PLGA microspheres showed negligible absorbance at 272 nm and 265 nm.

The surface morphology of 50% loaded particles was examined using a JEOL Model 6320 FV field emission scanning electron microscope (provided by the Massachusetts Institute of Technology Department of Materials Science and Engineering Electron Microscopy Center). Particles were mounted onto stubs and coated with a layer of gold/palladium. Samples were scanned at a voltage of 5kV at a probe current setting of 3 and working at a distance of 7 millimeters.

***In Vitro* Release of Bupivacaine and Lidocaine from Microparticles**

Fifty milligrams of PLGA microspheres were suspended in 1 mL phosphate-buffered saline, pH 7.4, at 37°C and inserted into the lumen of a Spectra/Por 1.1 Biotech Dispodialyzer (Spectrum Laboratories, Ranchodominguez, CA) with an 8,000 molecular weight cutoff. 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 a test tube with fresh phosphate-buffered saline. The bupivacaine and lidocaine concentrations in the dialysate were quantified by spectrophotometric means.

Cell Culture

All cell culture supplies were purchased from Invitrogen (Carlsbad, CA) unless otherwise noted. C2C12 mouse myoblasts (American Type Culture Collection CRL-1772, Manassas, VA) were cultured to proliferate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum and 1% penicillin streptomycin. Cells were then plated in 96 well tissue culture plates with 50,000 cells/mL/well in DMEM, supplemented with 2% horse serum and 1% penicillin-streptomycin, and allowed to differentiate into myotubes for 10–14 days. During differentiation the media were exchanged every 2–3 days. Cells were maintained at 37°C in 5% CO₂ with the remainder being ambient air. The hydrochloride salt of bupivacaine or lidocaine was added to DMEM at a concentration of 0.125% (w/v) and serially diluted to prepare the remaining concentrations.

Cell Viability Assay

To quantitatively assess cell viability after adding drug-containing media, a colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium [MTS] kit, cat. no. G358A; Promega Biosciences, CA, USA) was performed at predetermined time points. In live cells, the yellow tetrazolium salt is metabolized to purple formazan crystals and the color then quantified by spectrophotometric means. Absorbance of each well was measured at 485 nm using a Synergy Mx multi-mode microplate reader (BioTek Instruments, Inc., Winooski, VT).

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 United States National Research Council.

For all neurobehavioral and histologic studies, the observer was blinded to the type of particle injected.

Sciatic Blockade Technique

Nerve block injections were performed with a 20-gauge needle under brief isoflurane-oxygen anesthesia as described.²⁰ In brief, each rat was injected with 75 mg of microspheres suspended in 0.6 mL of 1% sodium carboxymethyl cellulose and 0.1% Tween 80 after gentle agitation. The needle was introduced posteromedial to the greater trochanter pointing in an anteromedial direction. Once bone was contacted, the particle-containing solution was injected.^{20,45}

Assessment of Nerve Blockade

The effectiveness of block was measured at predetermined time points, using methods previously described.^{8,20,45} Hindpaws were exposed in sequence (left, then right) to a 56°C hotplate (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.²⁰

Motor strength was assessed with a weight-bearing test. The animal was held over a digital balance allowing it to bear weight with one hindpaw at a time. The maximum weight that it could bear without its ankle touching the balance was recorded. The duration of motor blockade was defined as the time for weight bearing to return halfway to normal from maximal.

Tissue Harvesting and Histology

After euthanasia with carbon dioxide the sciatic nerve and surrounding muscle were harvested and processed to produce hematoxylin and eosin-stained slides, using standard techniques. A dissection score was given based upon the observed tissue reaction as follows: 0 = tissue planes obvious and easily separated; 1 = tissue planes obvious but separated with some difficulty; 2 = tissue planes effaced and separated with some difficulty; 3 = tissue planes completely obliterated, could not separate surrounding tissues from nerve without cutting through them.²¹ The samples were scored for inflammation (0–4) and myotoxicity (0–6).⁴² 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. Regeneration is characterized shrunken myocytes with basophilic cytoplasm. 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.

Statistical Analysis

Most data are reported as medians with 25th and 75th percentiles and are compared using the Mann-Whitney U test. This method was selected because the data were ordinal (dissection scores, inflammation scores, myotoxicity scores), or because they were not normally

distributed (neurobehavioral data). Results of MTS assays were described with parametric measures (mean, standard deviation, 1-way repeated measures ANOVA with Bonferonni's multiple correction test). LC₅₀ values were calculated using nonlinear least squares regression analysis of percent cell viability values. These tests were unpaired except when comparing sensory versus motor blockade in the same rat. To avoid type 1 error for gross dissection, inflammation and myotoxicity scoring, multiple comparisons were done in a planned manner (*i.e.*, comparisons were selected individually), and the *P* value required for statistical significance (α) was determined by dividing 0.05 by the number of comparisons. Therefore, for all 3 tissue scoring parameters (4 comparisons), $\alpha = 0.05/4 = 0.013$, so $P < 0.0013$ was required for statistical significance. For duration of blockade, there was only one comparison, so the α remained 0.05. We reported exact *P*-values.

Results

Cytotoxicity of bupivacaine and lidocaine

The myotoxicities of lidocaine and bupivacaine were compared *in vitro* using differentiated C2C12 myotubes.^{6,32,46} Myotoxicity was less for cells grown in media with lidocaine than with bupivacaine (Figs. 1a and b), at all durations of exposure for concentrations of 0.01% and larger ($n=8$, largest corrected $P < 0.050$). The LC₅₀ for bupivacaine (the concentration required to kill half of the cells) was lower than for lidocaine at all time points ($P < 0.00010$ for all time points)(Figure 1c).

Particle characteristics

Particles were produced containing 10 or 50% (w/w) lidocaine or bupivacaine (Table 1). Ten percent (w/w) and 50% (w/w) loaded microspheres were approximately 50 and 60 μm in diameter respectively. Particles examined by scanning electron microscopy were spherical (Figure 2).

Drug release from microspheres

Release of lidocaine or bupivacaine from 50 mg of 10% (w/w) or 50% (w/w) microspheres (Figure 3) was slowed compared to unencapsulated drug (1 mL of 0.5% (w/v) drug, or 5 mg). Bupivacaine release was slower than that of lidocaine. For example at 215 hours the 50% (w/w) bupivacaine particles had released 53.4 (50.6–57.2) % of their content compared to 74.2 (70.1–78.5) % for 50% (w/w) lidocaine particles ($p=0.032$). Similar trends were seen with the 10% (w/w) formulations.

Effect of drug selection on duration of sciatic nerve block

Animals received injections with 75 mg of particle at the sciatic nerve (Figure 4). Maximal sensory block (*i.e.*, a thermal latency of 12 sec) was achieved in 6 of 8 animals injected with 50% (w/w) lidocaine particles and 8 of 8 animals injected with 50% (w/w) bupivacaine particles ($p=0.13$, Chi-square). By 210 minutes, sensory block had resolved completely in the 50% (w/w) lidocaine group, whereas the 50% (w/w) bupivacaine group remained fully blocked. However, the median duration of thermal nociceptive block from 50% (w/w) lidocaine particles was 255 (90–540) min versus 840 (277–1215) min from 50% (w/w) bupivacaine particles; the difference was not statistically significant ($p=0.056$; $n = 8$).

To evaluate whether 50% (w/w) drug loading was so high that differences in quality of nerve blockade were obscured, eight animals were injected with 10% (w/w) loaded particles. The resulting duration of sensory block for rats injected with 10% (w/w) lidocaine was 15 (0–38) min ($n=4$). Only two met our definition of sensory nerve block (one lasting 30 minutes, the other 60 minutes). Of the four animals injected with 10% (w/w) bupivacaine particles, three developed sensory block lasting from 90–180 minutes with a median

duration 105 minutes (23–180). The difference in median durations of block between lidocaine and bupivacaine groups was not statistically significant ($p=0.37$).

To determine if formulations were sensory or motor selective, duration of sensory and motor blockade was compared. For 50% (w/w) bupivacaine particles the duration of sensory blockade was 840 (277–1215) min versus motor blockade of 570 (300–1140) min ($p=0.63$). 50% (w/w) lidocaine particles produced sensory blockade lasting 255 (90–540) min compared to motor blockade of 540 (420–870) min ($p=0.025$). Ten percent (w/w) bupivacaine particles resulted in 105 (23–180) min of sensory blockade versus 105 (60–165) min of motor blockade ($p=0.32$), and for 10% (w/w) lidocaine particles sensory duration was 15 (0–38) min compared to 30 (0–98) min ($p=0.51$). The durations of sensory and motor blockade of these formulations were not significantly different, except that in 50% (w/w) of lidocaine particles, motor blockade outlasted sensory blockade by 450 (330–728) min ($p=0.025$) (Figure 4).

Tissue Reaction

Groups of rats were injected at the sciatic nerve with 50% (w/w) lidocaine particles ($n=8$) or 50% (w/w) bupivacaine particles ($n=8$). The sciatic nerves were removed 4 days ($n=4$) or 2 weeks ($n=4$) after injection and processed for histology. Animals were also injected with 10% (w/w) lidocaine particles ($n=4$) or 10% (w/w) bupivacaine particles ($n=4$) to determine whether the high loading of drug in the 50% (w/w) loaded groups obscured differences in anesthetic effect or tissue toxicity. This was only done at 4 days, the time of maximal injury in the 50% (w/w) drug groups.

All injected rats had firm, white, globular deposits of particle in discrete pockets directly adjacent to the sciatic nerve (Figure 5), without spread to distant sites. The particle deposits appeared similar at 4 days and 2 weeks. The tissues immediately adjacent to the particle mass were adherent to the residue, but the latter was clearly separate from both muscle and nerve, i.e., there was no evidence of intraneural or intramuscular injection (on gross dissection or on subsequent microscopic examination), and tissue planes were easily identified and separated. Gross appearance was similar for all particle formulations and durations of exposure. On histologic examination, there was evidence of inflammation and myotoxicity in all animals (Table 2, Figure 6).

Tissue reaction at 4 days—Tissue reaction was evaluated in all groups at 4 days after injection, when inflammation and tissue injury is the most intense.²¹ On necropsy, gross appearance was similar between groups, as reflected in the lack of difference in dissection scores irrespective of drug or drug loading. On histological examination, the cellular response for all formulations was characterized by inflammatory cells consisting of neutrophils, lymphocytes and macrophages. PLGA microspheres were visible as round structures 40–60 μm across. There was evidence of myotoxicity in all animals (Figures 6a–d), characterized by shrunken myofibers with basophilic cytoplasm, in the cell layers proximal to the microspheres. Muscle injury occurred in a perifascicular pattern, worse in areas adjacent to pockets of particle residue, indicating extrinsic tissue injury originating from the particles. Deeper layers demonstrated edematous cells with centralized nuclei (i.e., a lesser degree of myotoxicity). There was no statistically significant difference in the inflammatory or myotoxicity scores between particles loaded with lidocaine or bupivacaine at either concentration (Figure 7; Table 2).

Particle drug loading did not influence inflammation or myotoxicity scores (Figures 7a and c). The median inflammation and myotoxicity scores for 10% (w/w) lidocaine particles were 1.0 (1.0–1.2) and 1.2 (0.9–1.7) respectively, compared to 3.0 (2.1–3.5) and 3.4 (2.1–4.2) for 50% (w/w) lidocaine particles (smallest $p=0.30$). For 10% (w/w) bupivacaine particles the

inflammation and myotoxicity scores were 1.4 (1.2–1.6) and 1.7 (1.5–1.9) respectively, compared to 2.7 (2.5–2.9) and 3.3 (2.9–3.5) for 50% (w/w) bupivacaine particles (smallest $p=0.029$). These differences were not significant by our predetermined α of $p<0.013$.

Tissue reaction 2 weeks after injection—For the 50% loaded particles, in which tissue reaction had been more severe, we followed tissue reaction out to 2 weeks after injection. The acute inflammatory reaction had been replaced by a chronic reaction with macrophages and lymphocytes. The microspheres were surrounded by multinucleated foreign-body giant cells. In all samples, adjacent muscle showed myofiber regeneration in more advanced stages than was observed in the 4-day cohort. In the layers close to the microspheres, regenerating myofibers remained shrunken, but demonstrated normal chromicity. Deeper myofibers had normal morphology with nuclei located at the periphery of the cell (Figures 6e–h).

The cellular inflammatory infiltrate resulting from both drug formulations was less severe and did not involve as much of the muscle fascicle as noted on day 4 (Figure 6g and h). For 50% (w/w) bupivacaine particles, the median inflammation scores decreased from 2.7 (2.5–2.9) on day 4 to 0.7 (0.5–1.1) at two weeks, and the myotoxicity scores decreased from 3.3 (2.9–3.5) to 1.7 (1.3–1.9) (smallest $p<0.029$). For 50% (w/w) lidocaine particles, the median inflammation scores decreased from 3.0 (2.1–3.5) on day 4 to 1.4 (1.3–1.6) at two weeks, and the myotoxicity scores decreased from 3.3 (2.9–3.5) to 1.7 (1.3–1.9) (smallest $p=0.34$). These differences were not significant by our predetermined α of $p<0.013$ (Figures 7b and d).

There were no statistically significant differences in the low inflammation or myotoxicity scores between the lidocaine and bupivacaine formulations at two weeks (Table 2). The inflammation scores for 50% (w/w) lidocaine particles were 1.4 (1.3–1.6) versus 0.7 (0.5–1.1) for 50% (w/w) bupivacaine particles ($p=0.10$). The myotoxicity scores were 1.9 (1.8–2.4) compared to 1.7 (1.3–1.9) for bupivacaine particles ($p=0.23$).

Conclusions

The key question addressed in this work, from a practical pharmaceutical point of view, was whether the selection of local anesthetic with respect to myotoxicity affects tissue injury from a controlled release system. Our results, using a very common, well established drug delivery vehicle, suggest that it does not. One might have expected the lidocaine microspheres to result in less severe tissue injury than bupivacaine, based on previous *in vivo* research documenting differences in the myotoxic potential of these two drugs,³⁰ and based on some reports using sustained-release vehicles.²⁵ Furthermore, our myotoxicity experiments with a mouse myoblast cell line (C2C12) demonstrated bupivacaine to be more toxic than lidocaine. These findings were similar to previous research, where bupivacaine was documented to be approximately 7-fold more cytotoxic than lidocaine.⁴⁶ In contrast, in PLGA microspheres, the choice of drug had little influence on the severity of inflammation and myotoxicity. This lack of difference may have been due to the relatively high concentrations that may have been obtained in the first few hours, and/or from long duration of exposure, both of which can have marked effects on toxicity.⁶ (Note that the concentrations used for *in vitro* toxicity were well below what is used clinically [0.25–0.75% for bupivacaine and 0.5–2% for lidocaine] since clinical concentrations caused immediate cell death [data not shown]). Such high concentrations might have been sufficiently toxic with both drugs that no difference could be seen. However, this lack of difference in toxicity was noted even in the 10% (w/w) loaded particles, in which there was relatively minimal nerve blockade (and therefore presumably not very high drug levels). By way of comparison, 0.5% (w/v) bupivacaine reliably yields a nerve block lasting

approximately 150 min,^{19,47} with minimal tissue toxicity.^{42,48} These observations are not consistent with the lack of difference in toxicity being simply due to excessive levels of local anesthetic.

We have previously noted that the mere presence of a sustained release vehicle can exacerbate local tissue toxicity^{19,42} *in vivo*, even though the vehicle might have no effect on local anesthetic cytotoxicity in cell culture.⁶ The mechanism for this effect is unknown. It is possible that the inflammation caused by the particles, or the particles themselves, worsens the severity of myotoxicity from agents that would otherwise be mild.^{6,21,37} Particles alone can also cause inflammation at the nerve that can considerably outlast the duration of blockade.^{21,41,43} The specific nature of the delivery vehicle itself may therefore have an impact on local tissue injury from local anesthetics. As with myotoxicity, the severity of inflammation appeared to correlate with drug loading (Figure 7a) and decrease over time.

Conventional local anesthetics are also neurotoxic.^{32,38,39,49} There are reports of bupivacaine-containing PDLA formulations where no neurotoxicity was documented.^{1,32} However, as noted in the introduction, neurotoxicity was a major factor leading to withdrawal of the Investigational New Drug application (IND#53,441)⁴⁴ of a sustained-release bupivacaine-dexamethasone formulation.³ The hematoxylin-eosin staining used here was not sufficiently sensitive to detect any but severe nerve injury. Nonetheless, because amino-amide (and amino ester) local anesthetics cause injury to both tissues in a concentration-dependent manner, the presence of myotoxicity suggests the potential for neurotoxicity.

As noted in the Results section, dissection revealed the presence of microparticulate residue immediately adjacent to the sciatic nerve. This observation would suggest that block durations as presented here are likely reflections of the local anesthetic capabilities of the microparticulate formulations rather than operator error (failed blocks). The lack of statistically significant difference in the durations of block from bupivacaine and lidocaine microspheres may seem surprising given that lidocaine solution provides shorter nerve blocks than bupivacaine.^{6,49,50} This lack of difference may, in part, have arisen from the relatively short duration of effect of the drugs used here compared to the timeframes over which the microspheres can release drugs. (Note the extremely long durations of block that can be achieved with these microspheres using synergistic drug combinations).³⁷ The great variability in nerve block durations from polymeric microsphere formulations may also have made differences hard to detect. That variability was consistent with our prior experience with PLGA microspheres for nerve blockade.^{6,20,37,43}

It is interesting to consider the risk of PDLA-related local toxicity in the context of ultrasound-guided regional anesthesia, which is now common practice. In many circumstances, ultrasound-guidance may allow PDLA formulations to be deposited in locales where they are adjacent to nerves but remote from major muscle groups. There will also be circumstances where that is not possible. It would remain to be seen how far muscle groups need to be from such devices to be unaffected. Of course, ultrasound guidance is not perfect: there is always the possibility of inadvertent injection into or near muscle. Many PDLA formulations could be used for infiltration anesthesia or field blocks, in which case ultrasound guidance would likely not be used, even in areas near muscles (perianal procedures, hernia repairs, etc.). It also bears emphasizing that one principal virtue of ultrasound-guidance, that it can deposit anesthetics right on the nerve, also has the potential to increase the probability of one of the most serious potential complications of PDLA: nerve injury.

We have observed myotoxicity with a wide range of delivery systems with very different compositions of matter, including PLGA,^{6,21,37,51} lipid-sugar-protein particles,^{21,41,43,51} polysaccharide-based gels,^{19,52} and thermosensitive nanogels.⁵³ This has raised the concern that myotoxicity may be an unavoidable consequence of increased concentrations or prolonged exposures to conventional local anesthetics, regardless of drug selection.⁶ The observation that even particles with low drug loading (10% in this study) and little anesthetic benefit generate myotoxicity is consistent with that view. Given the potentially severe tissue toxicity (muscle and nerve injury) that has been documented with sustained release of local anesthetics,⁴⁴ these results suggest that caution is warranted in the application of sustained release formulations of all amino-amide and amino-ester local anesthetics, particularly around muscle and nerve.

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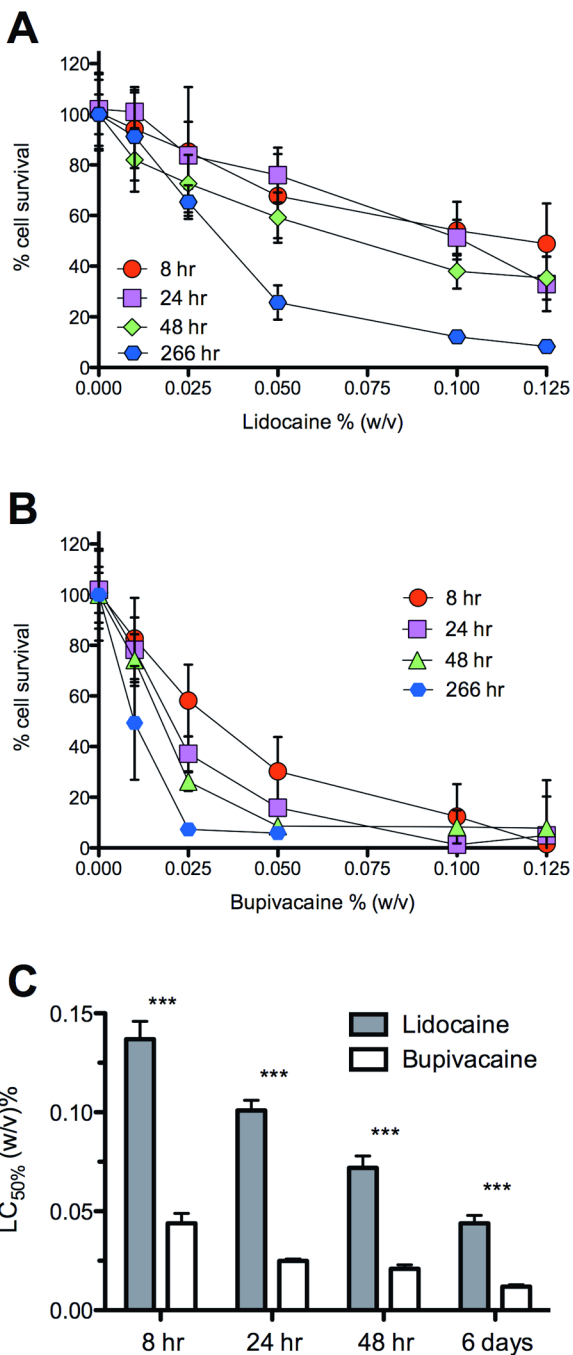


Fig. 1. Percentage of C2C12 cell survival after exposure to (A) free lidocaine or (B) bupivacaine for various durations. Also shown are the LC₅₀ values (C) derived from the percentage survival curves in (A) and (B). The LC₅₀ values are significantly lower for bupivacaine (***) for all time points, largest $P < 0.00010$). Data are means \pm standard deviation.

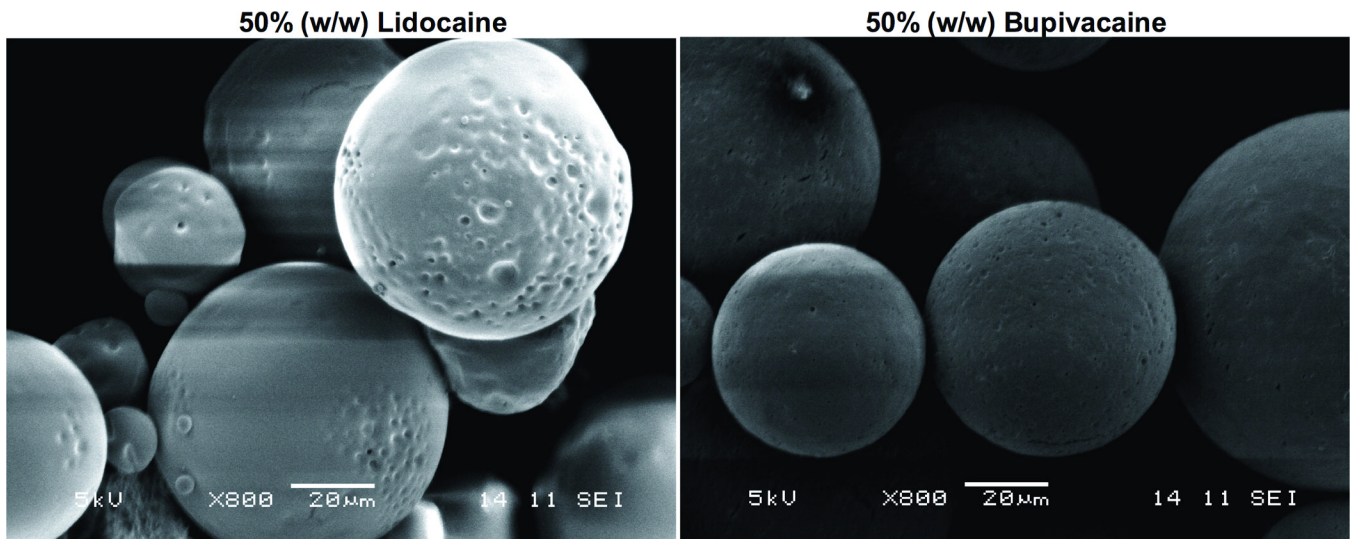


Fig. 2. Scanning electron micrograph of microspheres (50% (w/w) drug loading). Scale bar is 20 μm in both panels.

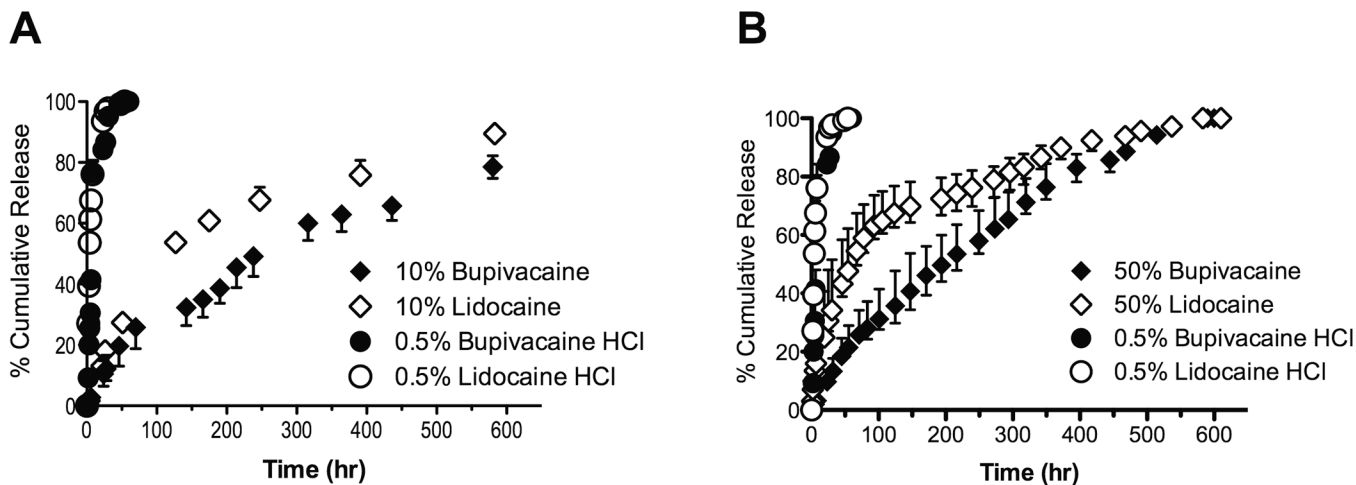


Fig. 3. Cumulative release of encapsulated bupivacaine or lidocaine eluted from (A) 10% (w/w) or (B) 50% (w/w) drug loaded microspheres. Also shown is the release of unencapsulated 0.5% (w/v) lidocaine or bupivacaine. Data are medians with 25th and 75th percentiles.

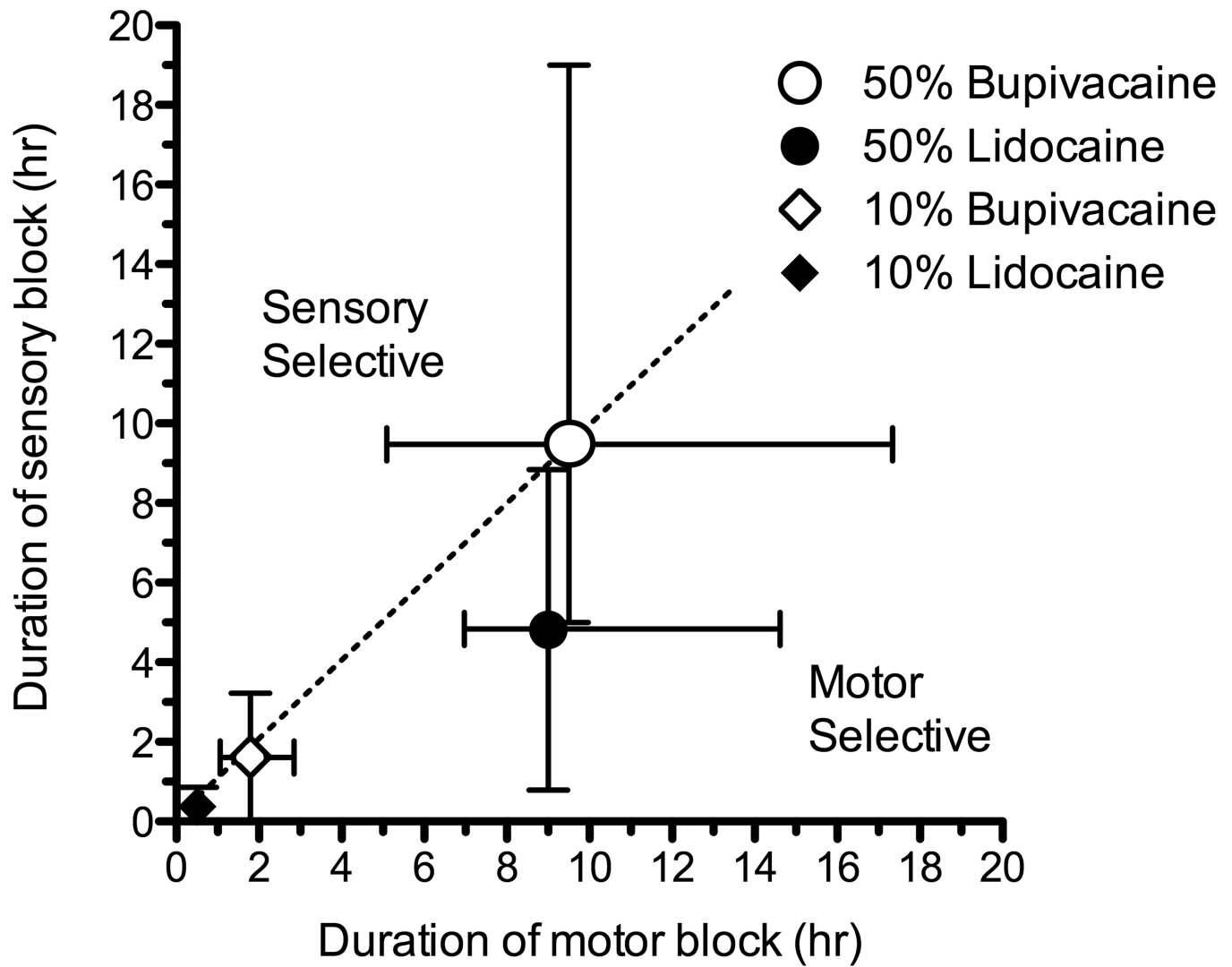


Fig. 4. Duration of sensory versus motor blockade after injection with local anesthetic-containing microspheres. Percentages are drug loading (w/w). Data are medians with 25th and 75th percentiles.

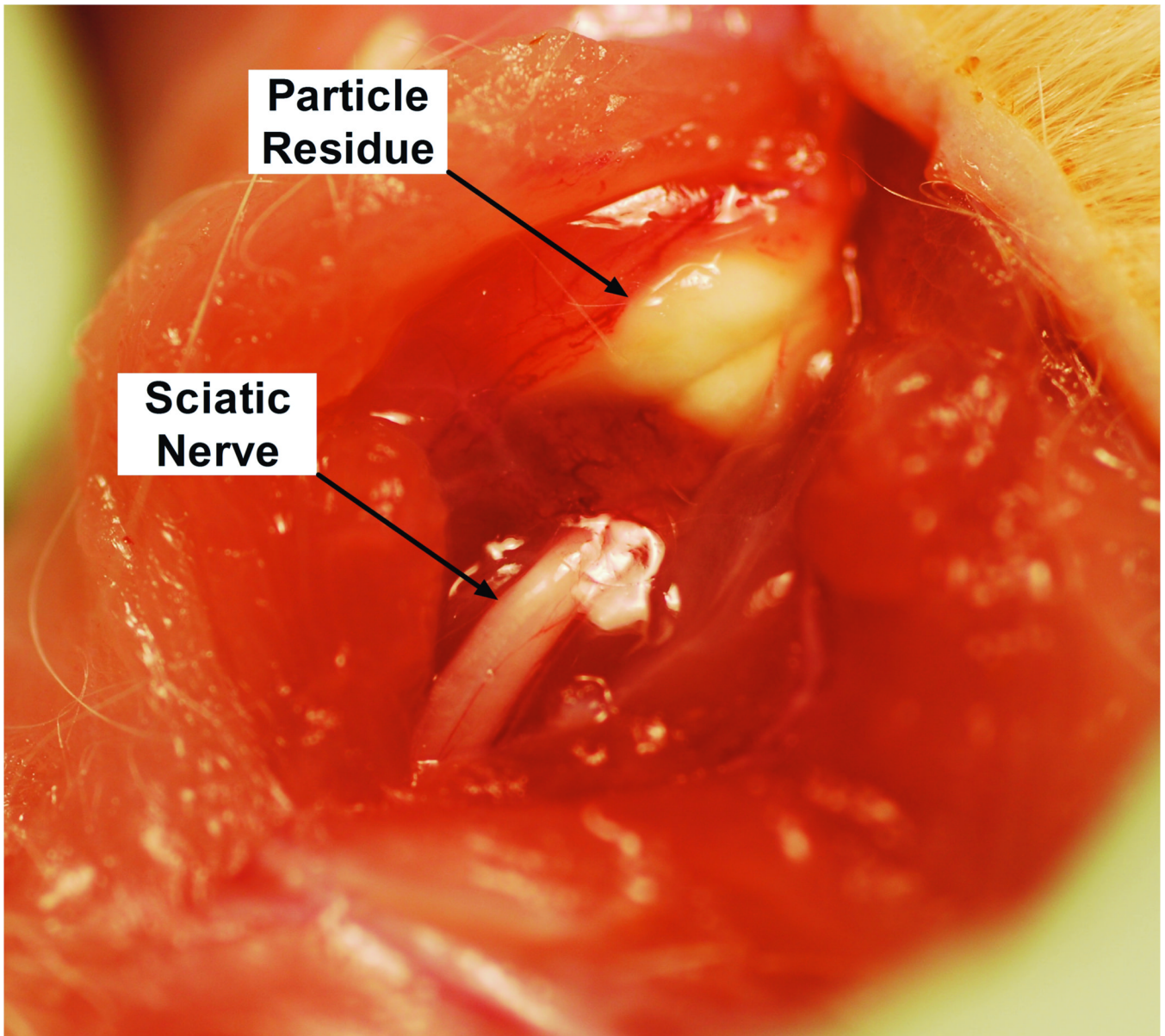


Fig. 5. Representative photograph of particle residue (here, with 50% (w/w) bupivacaine microspheres 4 days after injection). Residue appearance was similar in all particle formulations and time points.

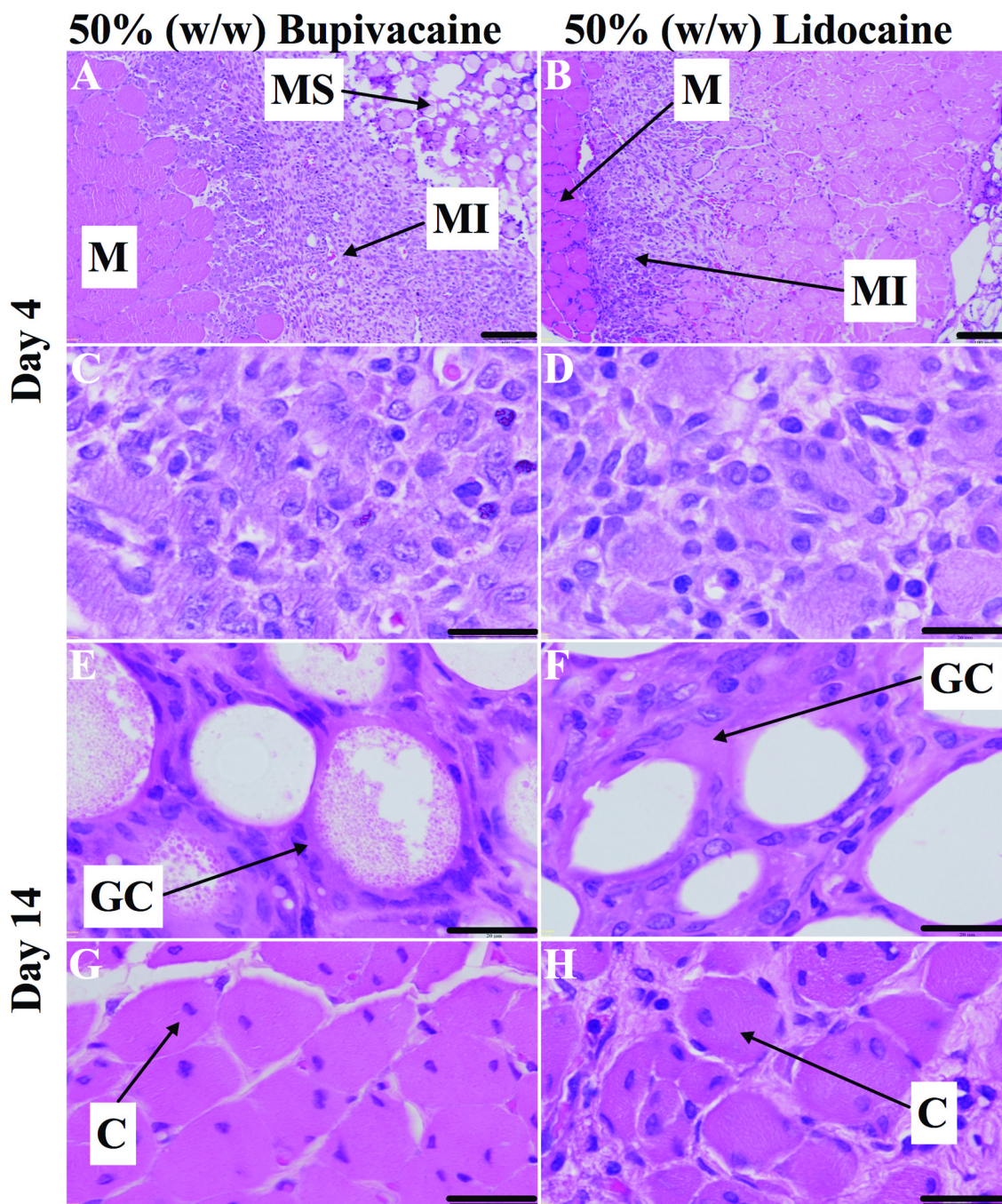


Fig. 6. Representative light microscopy of hematoxylin/eosin-stained sections of muscles (M), and surrounding tissues at the site of injection of 50% (w/w) bupivacaine or lidocaine loaded poly-lactic-co-glycolic acid (PLGA) microspheres (MS). (A and B) 4 days after injection, muscle injury (MI) extends deep into the muscle fascicle. (C and D) Tissue reaction was characterized by myocyte injury with abundant macrophages and occasional lymphocytes. (E and F) 2 weeks after injection, microspheres were surrounded by lymphocytes, macrophages and foreign-body giant cells (GC). (G and H) Tissue reaction was characterized by myocytes with nuclear centralization (C) surrounded by sparsely distributed lymphocytes and macrophages. Scale bars 100 μ m (A and B), 20 μ m (C–H).

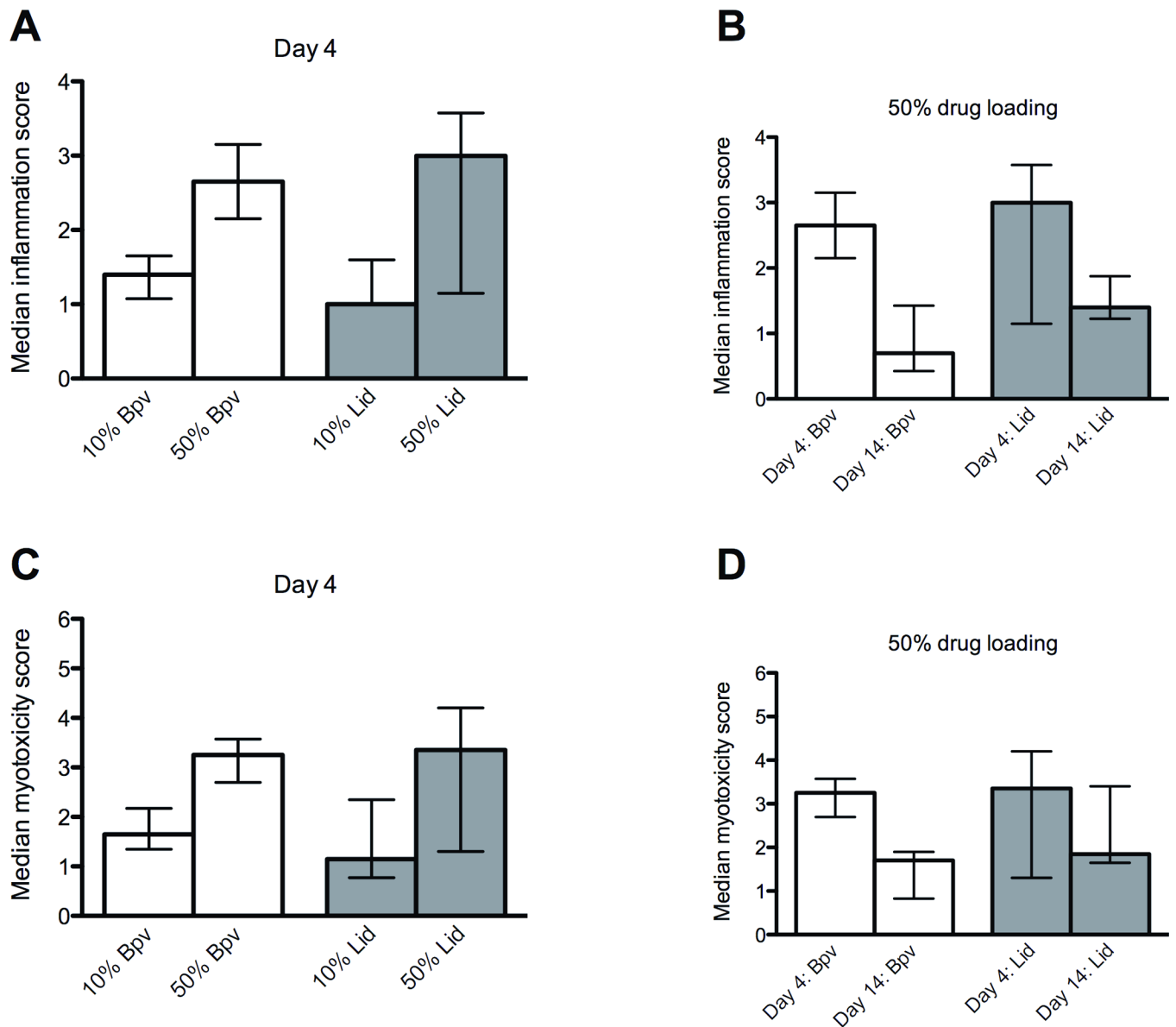


Fig. 7. Comparison of the effect of drug loading (A and C) and time after injection (B and D) on inflammation and myotoxicity. Data are medians with 25th and 75th percentiles.

Table 1

Characteristics of poly-lactic-co-glycolic acid (PLGA)-based microspheres

Microsphere drug content (w/w) ^a	n	Diameter (μm) ^b	Drug content (%) ^{b, c}
Lidocaine 10%	4	47.5 ± 13.9	10.0 ± 2.4
Bupivacaine 10%	4	49.2 ± 14.4	13.4 ± 2.1
Lidocaine 50%	4	65.2 ± 20.7	54.0 ± 3.0
Bupivacaine 50%	5	57.8 ± 16.3	51.7 ± 3.7

^aTheoretical^bValues are means ± standard deviations^cMeasured

Table 2

Values for the three measures of biocompatibility for each microparticulate formulation

Scale		Score	
		Day 4	Day 14
Gross dissection (0–3)	Lidocaine 10%	1.0 (0.8–1.0)	--
	Bupivacaine 10%	1.0 (1.0–1.3)	--
	<i>P value</i> *	0.13	
	Lidocaine 50%	1.0 (1.0–1.3)	1.0 (1.0–1.5)
	Bupivacaine 50%	2.0 (1.8–2.0)	2.0 (2.0–2.3)
	<i>P value</i> *	0.12	0.11
Inflammation (0–4)	Lidocaine 10%	1.0 (1.0–1.2)	--
	Bupivacaine 10%	1.4 (1.2–1.6)	--
	<i>P value</i> *	0.27	
	Lidocaine 50%	3.0 (2.1–3.5)	1.4 (1.3–1.6)
	Bupivacaine 50%	2.7 (2.5–2.9)	0.7 (0.5–1.1)
	<i>P value</i> *	0.44	0.10
Myotoxicity (0–6)	Lidocaine 10%	1.2 (0.9–1.7)	--
	Bupivacaine 10%	1.7 (1.5–1.9)	--
	<i>P value</i> *	0.19	
	Lidocaine 50%	3.4 (2.1–4.2)	1.9 (1.8–2.4)
	Bupivacaine 50%	3.3 (2.9–3.5)	1.7 (1.3–1.9)
	<i>P value</i> *	0.44	0.23

Values are medians with 25th and 75th percentiles in parentheses; n = 4 for all groups

Particles with 10% loading were tested at 4 days only

* *P* value of Mann-Whitney U test comparing lidocaine to bupivacaine for equally loaded particles