Injectable microparticle–gel system for prolonged and localized lidocaine release. II. *In vivo* anesthetic effects

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Abstract: Current treatment protocols for postoperative pain are beset by either the short duration of the anesthetic effect or requirement of hospitalization of the patients. We reported herein a novel treatment by applying to the surgical site a biodegradable microparticle–gel system for prolonged and localized release of encapsulated anesthetic drugs. In a previous publication, lidocaine-loaded poly(D,L-lactic acid) microspheres were fabricated and their formulations were optimized. *In vitro* characterization of these lidocaine-loaded microspheres, however, revealed a shortcoming of this system; that is, microspheres tend to fuse physically. Fusion of the microspheres could hinder their clinical applications, as it would clog the needle. In this article, we demonstrated that fabricating microspheres with high molecular weight (~60 KDa) poly(lactic-*co*-glycolic acid) would increase the glass transition temperature of the microspheres after lidocaine loading, thereby increasing their mechanical stability and eliminating their fusion during storage. Such microspheres containing 31% (w/w) lidocaine in the presence or absence of 25% (w/v) poloxamer 407 gel were then evaluated *in vivo* by monitoring the sensory and motor functions of the rats after sciatic nerve block, using the previously established hot-plate and weight-bearing testing methods. Results showed that microspheres formulated with poloxamer 407 gel yielded the longest duration of sensory and motor block for a period of approximately 8.5 h, compared to 5 h by microspheres in saline, 5 h by lidocaine in poloxamer 407 gel, and 2 h by lidocaine in saline. This study suggests that the microsphere–gel system containing lidocaine could potentially be applied clinically to the treatment of postoperative pain. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 70A: 459–466, 2004

Key words: lidocaine; poly(glycolic-*co*-lactic acid); poloxamer; microspheres; anesthetic

INTRODUCTION

Due to the short duration of anesthetic effects provided by currently existing postsurgery pain treatment, we have explored the possibility of utilizing a sustained-release microsphere formulation to overcome such problems. In a previous poly(D,L-lactide) (PLA, MW ~20,000)-based lidocaine-loaded microsphere system was successfully fabricated. *In vitro* characterization of these microspheres showed a sustained release of lidocaine over a period of more than 4 days without the initial drug burst release.[1] In addition, these lidocaine-loaded microspheres displayed a reasonably rapid degradation kinetics (~4 weeks), which would suit for the clinical application. Despite promises, *in vitro* studies also revealed a shortcoming of this system that could hinder its clinical application; that is, microspheres displayed a strong tendency to fuse during storage, which would therefore clog the needle during administration of the microspheres. In this article, we demonstrated that by utilizing high molecular weight poly(lactic-*co*-glycolic acid) (PLGA, MW ~60,000 daltons) or PLA (~130,000 daltons) would resolve this problem. Because PLGA would degrade faster than PLA, it was used to fabricate the microspheres. In an attempt to further prolong the anesthetic effect, 25% of poloxamer 407 (PO407) solution was formulated with these PLGA microspheres, because the aqueous PO407 solution at concentrations

above 20% exhibited a temperature-dependent gelation behavior.[2] Therefore, the incorporated PO407 solution would remain in an aqueous state at 4°C during administration but change to a gel state at the surgical site when temperature was raised to 37°C, retarding lidocaine release from the microspheres. *In vivo* testing of these formulated PO407–PLGA microspheres was conducted using a sciatic nerve blocked rat model.[3] The sensory and motor functions of the rats were assessed by the hot-plate and weight-bearing tests, respectively. Comparison was made with formulations consisting of PLGA-based microspheres or PO407 gel.

MATERIALS AND METHODS

Materials

Poloxamer 407 (PO407), lidocaine hydrochloride, lidocaine (Lid), and sorbitan monooleate (Span 80) were purchased from Sigma (St. Louis, MO). Poly(D,L-lactic acid) (PLA, i.v. ~0.4, MW ~20,000 dalton) and poly(vinyl alcohol) (PVA, MW ~6,000 dalton and 80 mol % hydrolyzed) were obtained from Polyscience Inc. (Warrington, PA). Poly(D,L-lactic acid) (PLA, i.v. ~0.73, MW ~130,000 dalton) and poly(lactic-*co*-glycolic acid) (PLGA, i.v. ~0.75, MW ~80,000 dalton) were purchased from Alkermes (Cambridge, MA).

Preparation of lidocaine-loaded PLGA microspheres

Lidocaine-loaded microspheres were prepared using the oil/water solvent-evaporation method.[4] Briefly, 800 mg PLGA, 800 mg lidocaine (free base), and 120 mg Span 80 were added to 2 mL methylene chloride. The organic phase was stirred for 3 h to form a homogenous solution and then mixed with 10 mL of 0.75% (w/v) PVA aqueous solution. After vortexing for 30 s, the mixture was sonicated for 1 min to produce an oil/water emulsion. This emulsion was then added to 90 mL of 0.75% (w/v) PVA aqueous solution and after continuous stirring for 2 h to evaporate methylene chloride, the mixture was passed through a stainless steel sieve (pore size of ~300 μm) to remove large particles. The microspheres were washed twice with ddH$_2$O, collected by centrifugation at 4°C, freeze dried, and then stored under vacuum at 4°C.

Preparation of microsphere–gel formulations

The 25% (w/v) PO407 gel solution was prepared by dissolving an appropriate amount of PO407 in a cold 0.9% saline solution. To prepare 2% of lidocaine · HCl in PO407 gel, an appropriate amount of lidocaine · HCl was dissolved in 0.9% saline solution, and then cooled down to 4°C before the addition of an appropriate amount of PO407. Microsphere–PO407 gel formulation was prepared directly prior to use by mixing an appropriate amount of the microspheres with the PO407 gel solution at 4°C.

Characterization of microspheres

Microspheres were characterized by using scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) according to procedures described previously.[1] Briefly, microspheres were carbon coated and fixed by graphite on a stub before SEM observation.

In vitro lidocaine release

Samples were enclosed in dialysis bags (MW cutoff = 12,000 dalton) and then placed in 200 mL of 0.1 M phosphate buffer (pH 7.4). The buffer was shaken at 30 rpm and 37°C. At predetermined time points, 1 mL of buffer was withdrawn and assayed for the released lidocaine using HPLC according to the procedures described previously.[1]

Animal care

Young adult male Sprague-Dawley rats weighing 310–420 g each were obtained from Taconic Farms (Germantown, NY) and housed in groups in a 6 a.m. to 6 p.m. light–dark cycle. Animals care was in compliance with protocols approved by the Animal Care and Use Committee at the Massachusetts Institute of Technology, and with the Principles of Laboratory Animal Care published by the National Institutes of Health. Each rat was injected with its designated sample once only.

Sciatic nerve blockade, neurobehavioral testing, and data analysis

To prepare the sciatic nerve blocked animals, male Sprague-Dawley rats were injected with samples at the sciatic nerve according to an established procedure.[3] In brief, animals were anesthetized in isoflurane:oxygen, and samples were then injected through a 23-gauge needle introduced posteromedial to the greater trochanter and advanced in an anteromedial direction until bone was contacted.[5] Samples were maintained on ice until the time of injection because of the reverse thermal gelation property of PO407. The left leg of the rat was always used for blocks, with the right leg serving as a control. Unless otherwise stated, six rats were included in each experimental group.

Nociceptive block was assessed by a modified hotplate test, in which the hindpaws of the rat were exposed to a hotplate at 56°C and the time (thermal latency) until the rat withdrew its paws was recorded.[6] If the paw of the rat

remained on the hotplate over 12 s without self-withdrawal, it was then removed to avoid injury. The data for nociceptive block were reported in terms of thermal latency (a measure of the degree of analgesia) and duration of block; the later was defined as the time required for thermal latency to return to 7 s (i.e., 50% of maximal latency given a baseline of approximately 2 s).

Motor strength was assessed by allowing the animal to bear weight on one hindpaw at a time, and then by measuring the maximum weight generated without its ankle touching the balance. The duration of motor block was defined as the time for weight bearing to return halfway to normal from maximal block.

For each group of animals, the latency at any given time point and the duration of block were expressed as means ± standard deviations. Comparisons between groups were performed using the Student's t-test.

RESULTS

Preparation and characterization of the microspheres

In a previous article,[1] we demonstrated that sustained lidocaine release could be achieved by increasing the Lid/PLA (MW ~20,000) ratio, Span80/PLA ratio, and PLA concentration. However, microspheres employed in these studies showed a tendency to fuse automatically. To overcome this fusion problem, high molecular weight PLA (MW ~130,000) or PLGA (MW ~80,000) was used in the current investigation to fabricate the microspheres. These newly prepared microspheres were found to be relatively stable and remain separated. To confirm the hypothesis that fusion was caused by a reduction in T_g due to the plasticizing effect of lidocaine on PLA, T_g of the microspheres made by high MW PLA or PLGA polymers with and without lidocaine loading were examined using DSC (see Fig. 1). Surprisingly, both types of microspheres displayed an increased T_g in the presence of lidocaine in contrast to the decreased T_g observed previously for microspheres made with low MW PLA polymer.[1] The reason that the plasticizing effect of lidocaine was not observed with the high MW polymers remains unclear at this moment.

Because PLGA degrades much faster than PLA due to its amorphous structure, PLGA-based microspheres were used in current studies. The same procedures established previously in modifying the microsphere formulations[1] by utilizing the highest PLGA concentration, 15% Span80/PLGA ratio, and the highest possible lidocaine loading, was followed. The morphology of these microspheres was examined by SEM (see Fig. 2), and the size of most particles were in the range between 1 and 100 μm.
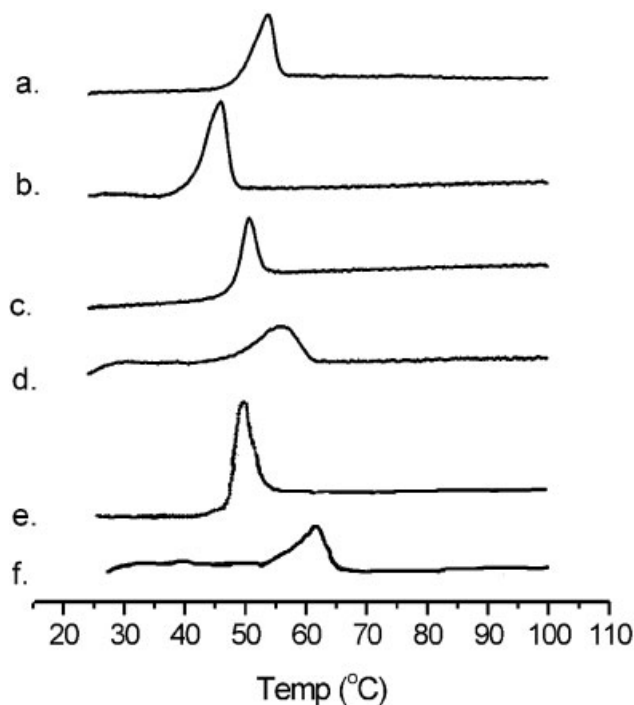

Figure 1. DSC diagrams of (a) PLA (MW ~ 20,000), T_g = 50°C; (b) PLA (MW ~20,000) of 11% lidocaine loading, T_g = 41°C; (c) PLA (MW ~130,000), T_g = 47°C; (d) PLA (MW ~130,000) with 27% lidocaine loading, T_g = 50°C; (e) PLGA (MW ~80,000), T_g = 46°C; and (f) PLGA (MW ~80,000) with 31% lidocaine loading, T_g = 54°C. T_g is defined as the temperature at the onset of the peaks.

In vitro release of lidocaine from various formulations

Lidocaine release from several different formulations was examined. As shown in Figure 3, lidocaine was released most rapidly from the formulation containing 1.3 mL of 2% (w/v%) lidocaine · HCl in 0.9% saline solution, followed by that containing 2% of lidocaine · HCl in 1.3 mL of 25% (w/v%) PO407 gel. Formulations containing 75 mg microspheres in 1.3 mL of 0.9% saline solution and in 1.3 mL of 25% (w/v%) PO407 gel exhibited the slowest lidocaine release. Microspheres used in the formulations contained 31% (w/w) lidocaine loading. Therefore, all the four studied formulations contained the same lidocaine dose of 23 mg (not counting the weight of HCl in lidocaine · HCl).

Effectiveness of the formulations on sciatic nerve block

Rats were injected at the sciatic nerve with one of four formulations (containing the same lidocaine dose of 23 mg): lidocaine · HCl (2%) in saline (0.9%); lidocaine · HCl (2%) in PO407 gel (25%); 75 mg microspheres (31% lido-
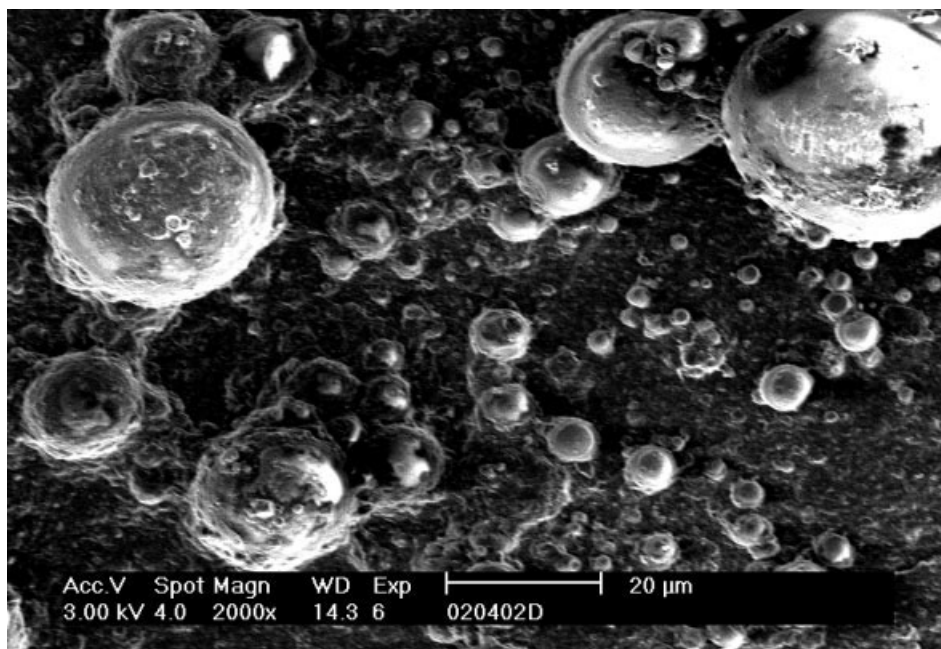
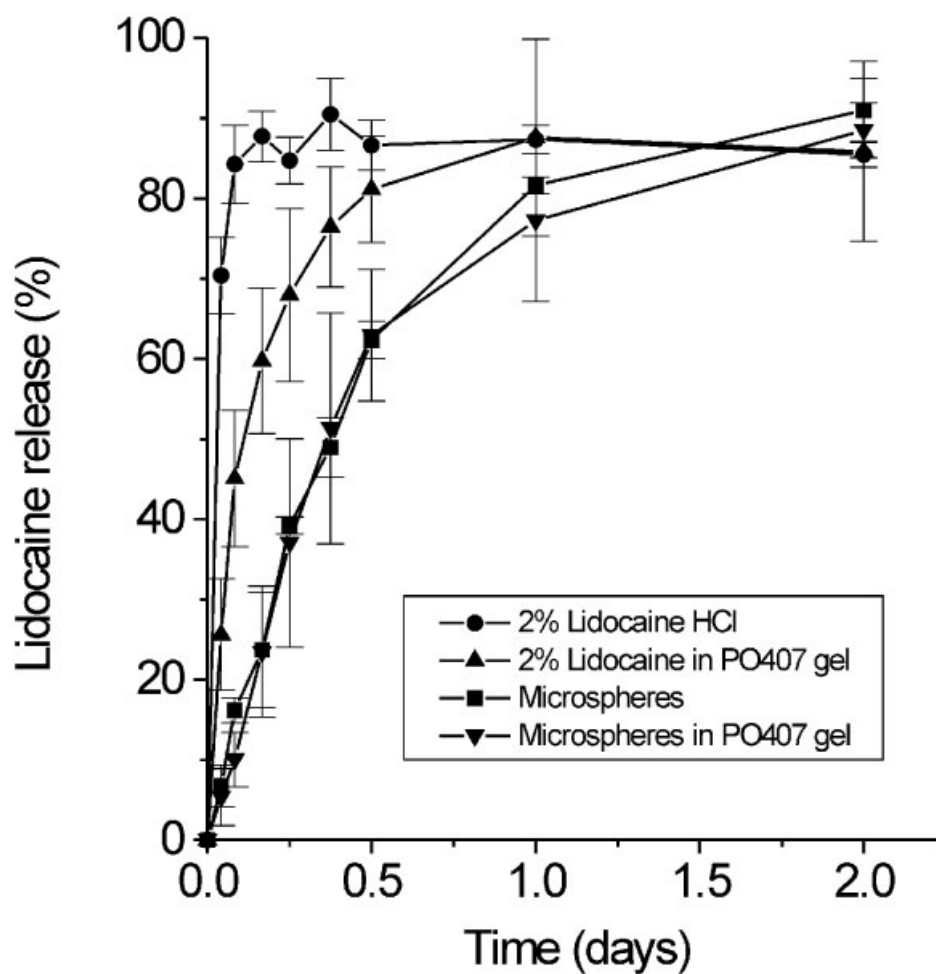**Figure 2.** Morphology observed by SEM of the PLGA microspheres containing 31% lidocaine.

Figure 3. *In vitro* release ($n = 3$) of (●) 2% lidocaine · HCl in 0.9% saline solution, (▲) 2% lidocaine · HCl in 25% (w/v%) PO407 gel, (■) 31% lidocaine microspheres in 0.9% saline solution, and (▼) 31% lidocaine microspheres in 25% PO407 gel.
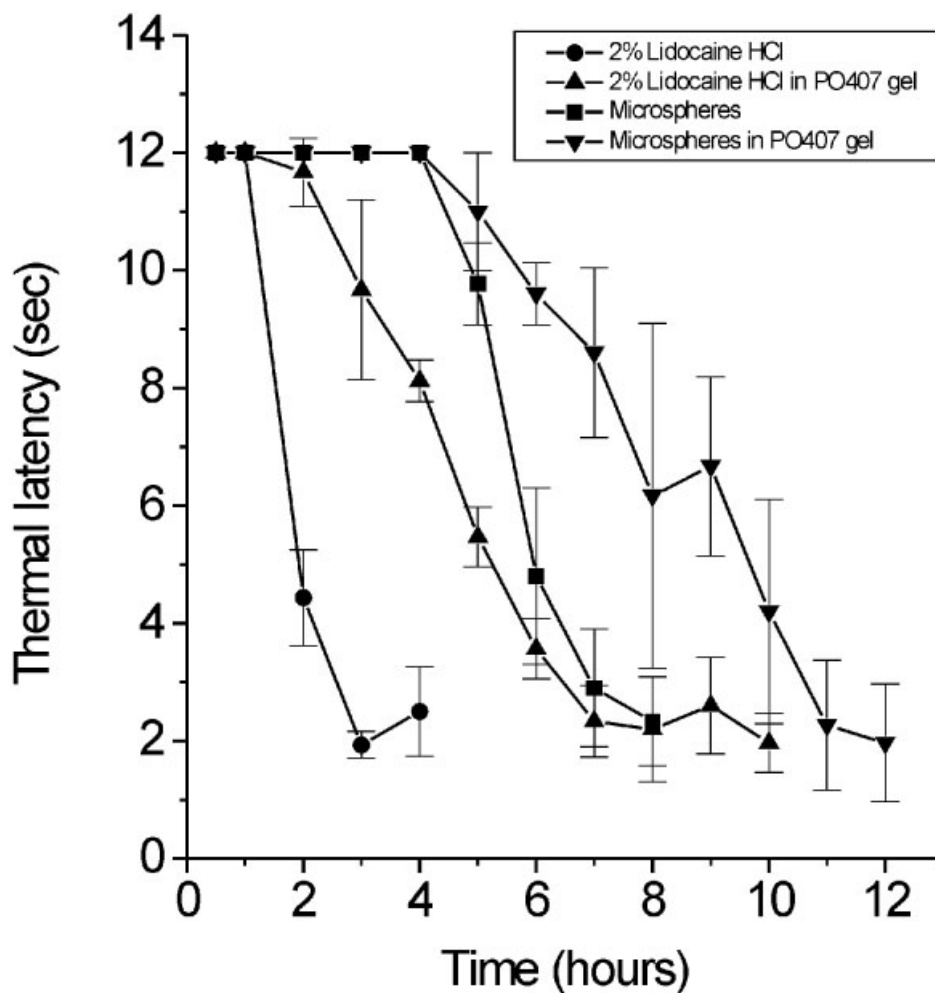
Figure 4. Changes of the thermal latency ($n = 6$) versus time of the leg injected with the four drug formulations; (●) 2% lidocaine · HCl in 0.9% saline solution; (▲) 2% lidocaine · HCl in 25% (w/v%) PO407 gel; (■) 31% lidocaine microspheres in 0.9% saline solution; and (▼) 31% lidocaine microspheres in 25% PO407 gel.

caine loading) in saline (0.9%), and 75 mg microspheres (31% lidocaine loading) in PO407 gel (25%). As shown by results in Figure 4, all rats who received one of the four different formulations achieved the maximal nerve block (thermal latency = 12 s) by the time of the first testing; that is, 30 min after sample injection. However, results in Figure 5 showed that lidocaine in PO407 gel yielded a longer duration of sensory (271 ± 21 min) and motor (421 ± 46 min) block than lidocaine in saline (126 ± 35 min and 124 ± 31 min for durations of sensory and motor block, respectively; $p < 0.001$). This finding suggested that the PO407 gel was able to slow the diffusion of lidocaine from the sciatic nerve. Although no statistically significant difference was observed ($p = 0.048$) in the duration of sensory block between lidocaine in PO407 gel (271 ± 21 min) and microspheres in saline (301 ± 25 min), the former nevertheless yielded a longer duration of motor block (421 ± 46 min) than the latter (341 ± 4 min) ($p = 0.001$). Among the four tested formulations, microspheres in PO407 gel displayed the longest duration of motor and sensory block (429 ± 35 min and

537 ± 36 min, respectively; $p < 0.001$ when compared to the formulation of microspheres in saline). To ensure that the nerve block resulted directly from the lidocaine, PO407 gel without lidocaine loading was also examined and found to produce no anesthetic effect.

Overall, motor blockade was longer than sensory blockade in most of the formulations by the following proportions: lidocaine in PO407 gel by 56% ($p < 0.001$), microspheres in saline by 13% ($p = 0.014$), microspheres in PO407 gel by 9% ($p = 0.003$), except for the formulation of lidocaine in saline by 0% ($p = 0.821$).

Systemic distribution of lidocaine

To assess the degree of systemic distribution of the drug from different formulations, all animals were tested for functional deficits in the leg that did not receive sample injection.[7] Figure 6 showed that rats injected with lidocaine in PO407 gel, microspheres in
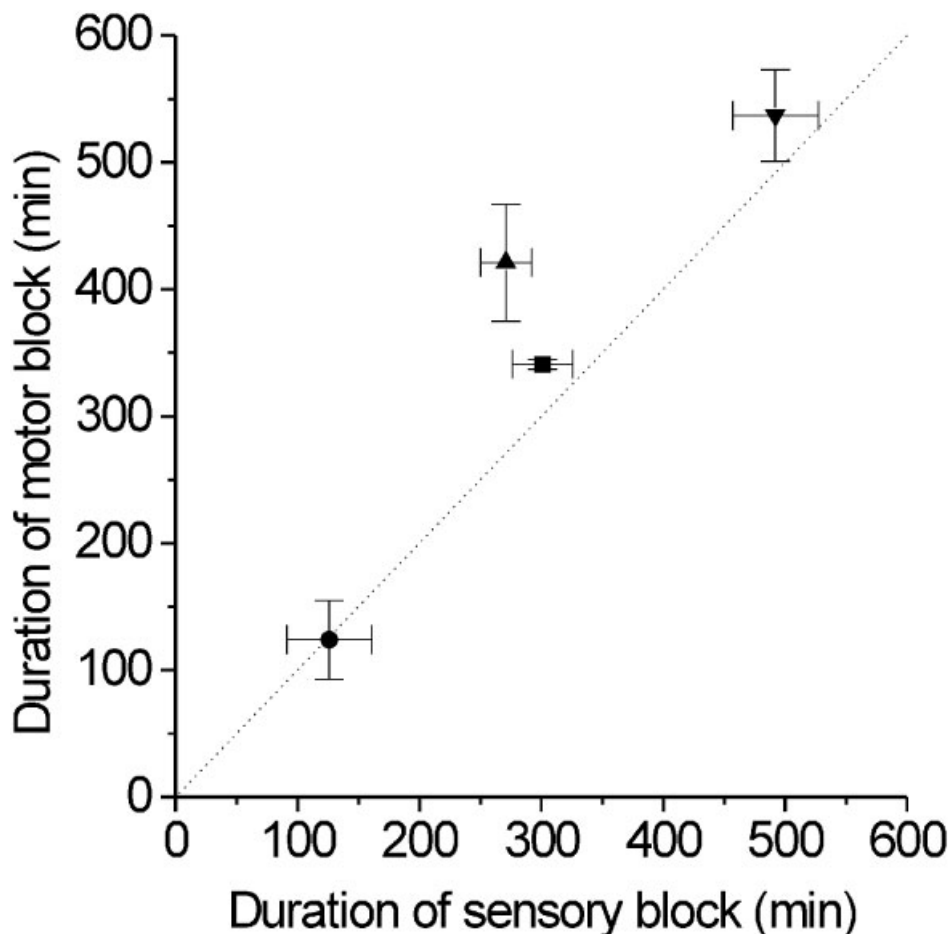
Figure 5. Comparison of durations of sensory blockade versus motor blockade ($n = 6$); (●) 2% lidocaine · HCl in 0.9% saline solution; (▲) 2% lidocaine · HCl in 25% (w/v%) PO407 gel; (■) 31% lidocaine microspheres in 0.9% saline solution; and (▼) 31% lidocaine microspheres in 25% PO407 gel. Points falling above the diagonal line bisecting the graph represent a relative motor predominance in nerve blockade, whereas those falling below the diagonal line indicate a sensory predominance.

saline, or microspheres in PO407 gel yielded a thermal latency of ~2 s (baseline) from beginning to the end, exhibiting either minimal or no contralateral deficits. These results suggested minimal systemic distribution of lidocaine. In contrast, rats injected with lidocaine in saline yielded a significant increase in latency in their uninjected leg at the time of the first observation (30 min), and such a functional deficit was gradually resolved over the next 2–3 h. In this regard, with only the exception of the formulation containing lidocaine in saline solution, the other three formulations all appeared to achieve significant nerve block with only minimal systemic distribution (i.e., less toxicity) of the delivered lidocaine.

DISCUSSION

As demonstrated in Figure 5, lidocaine in PO407 gel yielded a significantly longer duration of motor block than that of sensory block ($p < 0.001$) when compared

with the other formulations; a property that was not desirable because it would result in a paralyzed limb with full sensation. The most likely explanation for this phenomenon was that, after the majority of lidocaine was diffused away (when full sensation was recovered), the PO407 gel still contained the level of lidocaine that was sufficient enough to cause motor block but not sensory block. Indeed, the ability of PO407 gel to withhold lidocaine longer than the saline solution was seen from results in Figure 4, where it showed that it took longer (about 6–7 h) for the thermal latency induced by both PO407 formulations (i.e., lidocaine in PO407 gel and microspheres in PO407 gel) to drop from maximum (12 s) to minimum (2 s) than that (about 2–4 h) by both saline formulations (i.e., lidocaine in saline and microspheres in saline). The need of a lesser amount of lidocaine for motor block was because the large myelinated fibers (A fibers) that mediated motor function were more sensitive to amino-amide local anesthetics than were the small unmyelinated fibers (C fibers) that mediated pain. The slow diffusion of lidocaine in PO407 gel could also
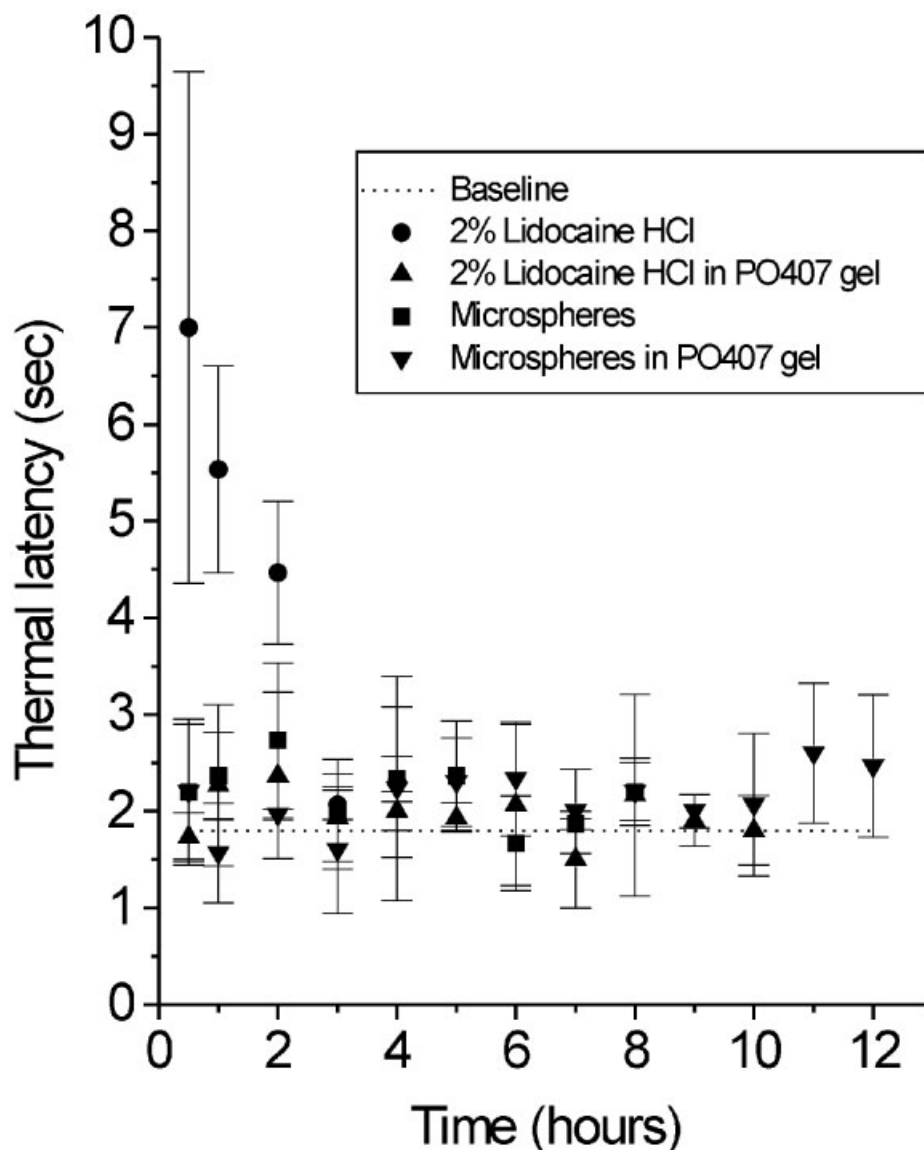
Figure 6. Changes of the thermal latency ($n = 6$) over time of the control leg (right leg) after the left leg was injected with the four formulations; (●) 2% lidocaine · HCl in 0.9% saline solution; (▲) 2% lidocaine · HCl in 25% (w/v%) PO407 gel; (■) 31% lidocaine microspheres in 0.9% saline solution; and (▼) 31% lidocaine microspheres in 25% PO407 gel.

account for the significantly longer motor block with lidocaine in PO407 gel formulation than microspheres in saline formulation, while both formulations yielded the same sensory blockade. On the other hand, motor block by lidocaine in saline did not last any longer than sensory block ($p = 0.821$); the result of a fast diffusion (or systemic distribution) of the delivered lidocaine.

Bupivacaine has been shown in clinical practice to have a slower onset and a longer duration of anesthetic effect than lidocaine. In a previous report,[8] it was observed that 4 out of 10 rats treated with PLGA microspheres containing a dose of 38 mg bupivacaine did not achieve the maximal nerve block (thermal latency = 12 s) by the time of the first measurement (30 min). In our study, however, all rats treated with microspheres containing a dose of 23 mg lidocaine

achieved the maximal nerve block within 30 min. These findings suggested that the lidocaine microspheres prepared in our study would immediately relieve patients from pain after surgery compared with the bupivacaine microspheres that would yield a slower onset even at a higher dose. Other investigators reported a 6-h duration of nerve block when treating the rats with PLGA microspheres containing 50 mg bupivacaine.[9] Comparing to their results, our formulation of microspheres containing only 23 mg lidocaine yielded a comparable duration of 5 h despite the fact that lidocaine possessed an intrinsically shorter duration than bupivacaine. Of greater promise is that microspheres in PO407 gel with only 23 mg lidocaine yielded an 8-h duration of nerve block.

Based on the assumption that the optimal lidocaine

dose for rat and human is weight proportional, 15 g microspheres would be needed for a 70-kg individual because 75 mg microspheres was used in the rat (0.35 kg in weight). A dose of more than 15 g microspheres would be both unrealistic and impractical in clinical practice. However, this assumption may not be correct judged by results from our animal studies. In the current study, 23 mg lidocaine solution was shown to achieve a 2-h anesthetic effect in rats, whereas in real clinical practice, only 100 mg lidocaine solution would be needed to achieve a 1–2 h anesthetic effect in humans.[10] Based on this observation, the dose difference between human and rat is only about 5–10 folds rather than the estimated 200 times. This discrepancy in dose could be due to the difference between localized drug delivery and systemic drug administration. In application of the microsphere–gel system, lidocaine was administered locally and in direct contact with the target nerve areas to provide an immediate effect, whereas the assumption of a weight-proportion in dose should only be applicable when the drug was administered systemically. Because only a 5–10-fold increase in dose would be required from rats to humans, 750 mg of the microspheres in PO407 gel would deem to be sufficient in yielding an anesthetic effect of longer than 8 h. In this regard, a substantially longer anesthetic effect should be observed if the microsphere dose is increased up to the maximal applicable dose of 15 g for humans.

In conclusion, sustained release of lidocaine from the PLGA microparticle–PO407 gel could clinically provide a prolonged anesthetic effect with a rapid onset yet without significant systemic toxicity. Efforts to further optimize this system to achieve a clinically relevant approach for effective postsurgical pain treatment are continued in our laboratories.

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