Long-term Effect of Sciatic Nerve Block with Slow-release Lidocaine in a Rat Model of Postoperative Pain

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ABSTRACT

Background: Postoperative pain management is important for preventing perioperative complications. The authors examined the effectiveness of controlled-release lidocaine for sciatic nerve block in a rat model of postoperative pain.

Methods: The authors created a novel slow-release lidocaine sheet (SRLS) with polylactic-coglycolic acid. In male Sprague-Dawley rats (postoperative pain model), the authors applied the SRLS, lidocaine alone, or polylactic-coglycolic acid (control) near the ipsilateral sciatic nerve just before making the paw incision. Mechanical hypersensitivity was assessed using von Frey filaments, and c-fos expression was examined in the spinal cord dorsal horn at segments L4-L5. Neurotoxicity and muscle toxicity were also evaluated via histopathology.

Results: The SRLS (30%, w/w) continuously released lidocaine for 1 week in vitro. The withdrawal threshold in the SRLS-treated group was higher than that in the control group at all time points measured (2 h to 7 days). The withdrawal threshold in the lidocaine-treated group was higher than that in the control group only at 2 h after paw incision.

Conclusions: Single treatment with the SRLS inhibited hyperalgesia and c-fos expression in the spinal cord dorsal horn for 1 week. Slow-release local anesthetics are promising for the management of postoperative pain.

The mean number of c-fos immunoreactive neurons in the SRLS-treated group was lower than in the control group at 2, 5, and 48 h after paw incision and lower than in the lidocaine-treated group at 5 and 48 h after paw incision. On histopathology, signs of inflammation were only slightly present in the muscle and nerve tissues of the SRLS-treated group.

What We Already Know about This Topic

- Slow-release preparations of local anesthetics would be ideal for regional analgesia after surgery
- Such preparations are limited by their low concentrations of local anesthetics

What This Article Tells Us That Is New

- Using a formulation of sheets of polylactic-coglycolic acid, we generated a 30% lidocaine concentration product, which reduced behavioral and neuronal evidence of postoperative pain for 1 week after incisional surgery in the rat

PAIN after surgery continues to be a major management challenge in clinical practice. A recent meta-analysis covering approximately 20,000 patients and 800 publications revealed that 41% of all surgical patients experience moderate to severe acute postoperative pain. Efficacious postoperative analgesia improves patient satisfaction, decreases morbidity, and reduces mortality after surgery. Postoperative pain management might also contribute to preventing cardiac events and pulmonary complications and decreasing medical expenses.

Neuronal block with local anesthetics is widely used for intraoperative and postoperative pain management. The duration of analgesia with a single injection of local anesthetics, however, is limited to approximately 4 to 8 h, even with long-acting local anesthetics such as bupivacaine and ropivacaine. Continuous infusions of local anesthetics via an indwelling catheter are often used for postoperative pain management, particularly when applied in the epidural space or...
at peripheral nerves. The insertion of epidural catheters has become increasingly difficult because of the increase in the number of patients taking anticoagulant or antithrombocyte medications for cerebral infarction, myocardial infarction, or prevention of pulmonary thromboembolism. In addition to neuronal blockade, several approaches have been used to reduce postoperative pain, including systemic opioids and nonsteroidal antiinflammatory drugs. These approaches, however, are associated with various side effects that limit their effective application.

The development of several slow-release techniques based on microspheres,4–6 microdroplets,7 and liposomes8,9 has made it possible to produce ultra long-acting local anesthetics. Microspheres are synthesized from polylactic acid polymer or a copolymer of polylactic-coglycolic acid (PLGA). In the current study, we generated slow-release lidocaine sheets (SRLS) from PLGA that release lidocaine over a period of 1 week to enhance postoperative pain management. The rat model of postoperative pain produced by hind paw incision is well established.10 This model demonstrates reproducible, quantifiable, and mechanical hyperalgesia that lasts from several days to a week after the incision. We applied the SRLS around the sciatic nerve in the rat model of postoperative pain and evaluated the efficacy of the treatment for hypersensitivity after paw incision.

Materials and Methods

Animals

This investigation was approved by the Animal Care and Use Committee of Gunma University Graduate School of Medicine (Maebashi, Japan). A total of 186 male Sprague-Dawley rats, weighing 250 to 300 g, were used in all experiments. Rats were allowed free access to food and water and maintained on a 12-h light–dark cycle. Sixteen rats were excluded from the study because of accidental death or surgical failure; data from 170 rats were included in the analysis.

SRLS Preparation

Lidocaine-loaded (30%, w/w) sheets (SRLS) were prepared by dissolving lidocaine (86 mg) and PLGA (200 mg; polylactic acid:polyglycolic acid = 50:50, molecular weight 85,000; Absorbable Polymer International, Pelham, AL) in 100% chloroform (2.2 g; density 1492 mg/ml; Sigma, St. Louis, MO), and then pouring the solution onto circular glass plates (48 mm diameter, approximately 1810 mm²). The lidocaine/PLGA/chloroform solution was incubated for 2 days at 25°C to allow the chloroform to evaporate, as it has been previously shown that evaporation occurs within 1–2 days when incubated in a hood at room temperature.11 We observed in the current study that when the solvent has evaporated, the sheets become easy to remove from the glass mold. The sheets obtained (286 mg) were clear (visual observation), indicating that the lidocaine was homogeneously distributed within the PLGA matrix. The thickness of the sheets was 200 μm. We cut the SRLS into small pieces, and the required amount of SRLS (based on calculated lidocaine dose) was implanted for in vivo experiments.

In Vitro Lidocaine Release Study

The SRLS was placed into a beaker filled with phosphate-buffered solution (pH 7.40). This buffered solution was made from monobasic sodium phosphate (12.69 g) and dibasic sodium phosphate (43.74 g) in 4 l of distilled water. The beaker was shaken in a 37°C water bath. We prepared two beakers in the same way and sampled 0.3 ml from one of the beakers at several time points and then made up the loss with the solution from the other beaker. The lidocaine concentration in each sample was measured by SRL Inc. (Maebashi, Japan) using a fluorescence polarization immunoassay.

In Vivo Lidocaine Release Rate Study

To estimate the amount of lidocaine released from the SRLS in rats, we measured the amount of lidocaine in the residual SRLS. We removed the residual SRLS from the administration site in rats after perfusion fixation at 2, 5, 10, 24, 48, and 72 h and 7 days after administration. The residual SRLS was placed in a beaker filled with phosphate-buffered solution (pH 7.40) and shaken in a 37°C water bath. We measured the lidocaine concentration in the beaker 2 weeks later.

Drug Application

This procedure was performed immediately before the paw incision. During isoflurane anesthesia (2% isoflurane in 100% oxygen), an incision was made from the left sciatic notch to the distal thigh. The subcutaneous tissue was bluntly dissected under the skin to expose the biceps femoris muscle. The sciatic nerve was freed from its investing fascia, and SRLS, lidocaine, or PLGA was administered near the sciatic nerve. Lidocaine (6 mg), SRLS (30%, 20 mg), or PLGA sheets (no lidocaine, 20 mg) were applied perineurally to the sciatic nerve. Lidocaine was administered as a powder. The SRLS (approximately 4 mm × 4 mm) containing 6 mg lidocaine was placed underneath the nerve. In another group, the SRLS was placed subcutaneously in the backs of rats to investigate the systemic effect of lidocaine released from the SRLS.

Postoperative Pain Model

The surgery was based on the procedure described by Brennan et al.10 In this model, a 1-cm incision was made on the plantar surface of the left hind paw during isoflurane anesthesia (2% isoflurane in 100% oxygen). The incision was started immediately distal to the heel and extended to a point just proximal to the first set of footpads. The plantaris muscle was elevated using forceps and incised longitudinally. The wound was closed with two mattress sutures using 5-0 silk. After surgery, the rats recovered from the anesthesia in their cages. Wounds were checked for evidence of dehiscence before behavioral testing.
Behavioral Testing

Rats were placed in individual plastic chambers with a plastic mesh floor and allowed to acclimate to the environment for 30 min. The mechanical withdrawal threshold was determined using calibrated von Frey filaments (Stoelting, Wood Dale, IL). The filaments were applied vertically to an area adjacent to the wound for 5–6 s with gentle bending of the filament. Withdrawal of the hind paw from the stimulus was scored as a positive response. The tactile stimulus producing a 50% likelihood of withdrawal threshold was determined using the up–down method, as described previously.12

Motor weakness in the affected paw was evaluated based on the motor impairment score as described previously.13 Score 0 represented the normal condition: the rats were able to walk and grasp normally. Score 1 represented partial blockade: when walking, the rats walked, gathered the forepart of the foot, kept it sideways, and had limited ability to grasp the bars. Score 2 represented severe blockade: the rats dragged their leg and failed to grasp the bars on elevation of the hindquarters. All behavioral studies were performed in a randomized, blinded manner.

Plasma Lidocaine Concentration Measurement

The serum concentration of lidocaine was measured at 2, 5, and 10 h after the administration. During pentobarbital anesthesia before perfusion for fixation, 3 ml blood was sampled and then centrifuged. The serum samples were kept at −80°C. The concentration of lidocaine in the serum was measured by SRL Inc., using a fluorescence polarization immunoassay.

C-fos Protein-like Immunoreactivity

Immunohistochemical detection of fos-like immunoreactivity was performed as described previously.14 A subset of animals from each treatment group was deeply anesthetized with intraperitoneal pentobarbital (100 mg/kg) at one of several time points postsurgery and perfused through the aorta with 4°C 0.01 M phosphate-buffered saline containing 1% sodium nitrite, followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline. The spinal cord was dissected out, postfixed in the same fixative for 3 h, and cryoprotected by immersion in 0.1 M phosphate buffer containing 30% sucrose at 4°C. Three days later, the spinal cord segments containing L4–L5 were cut transversely into 40-μm sections using a cryostat.

Immunohistochemistry was performed on the free-floating spinal cord sections. The sections were rinsed four times in 0.01 M phosphate-buffered saline containing 0.3% Triton X-100, incubated in 0.3% hydrogen peroxide for 15 min, and washed four times in 0.01 M phosphate-buffered saline containing 0.3% Triton X-100. The tissues were dehydrated by incubation in 50% ethanol for 45 min and then washed four times in 0.01 M phosphate-buffered saline containing 0.3% Triton X-100. After incubation in 1.5% normal goat serum (S-1000; Vector Laboratories, Inc., Burlingame, CA) for 1 h, the sections were incubated overnight at 4°C with c-fos antibody (1:20,000 in 1.5% normal goat serum). The sections were washed twice in 0.01 M phosphate-buffered saline containing 0.3% Triton X-100, and then incubated for 1 h each in goat antirabbit biotinylated secondary IgG complex (1:200; in 1.5% normal goat serum; BA-1000 Vector Laboratories, Inc.) and ABC complex (1:100, Vectastain ABC-Elite kit; Vector Laboratories Inc.). The reaction product was visualized with diaminobenzidine. The sections were mounted on glass slides, dried, and coverslipped. Images were captured on an OLYMPUS BX41 (Olympus Co., Tokyo, Japan) microscope using a QIMAGING MicroPublisher camera and QCapture software (VayTek, Inc., Fairfield, IA). To count the c-fos-positive neurons, the dorsal horn portion of the images was divided into three regions, including the superficial layer (laminae I-II), nucleus proprius (III–IV), and deep dorsal horn (V–VI) based on the cytoarchitectonic organization of the spinal cord.15 We determined the segmental level of the tissue section using light field microscopy at 10× magnification and counted each stained c-fos immunoreactive neuron manually at 200× magnification for each of the three layers described earlier. The six sections of the L4–L5 spinal cord from each rat were randomly selected for quantification of c-fos-positive neurons. The number of c-fos immunoreactive neurons for one rat was obtained as the mean of six L4–L5 sections. In each group, the number of c-fos immunoreactive neurons was obtained as the mean score of six rats. Counting of c-fos-positive neurons was performed by an investigator blinded to the drug treatment.

Histopathology

For the assessment of histopathology, we excised the sciatic nerves 1 week after drug administration. Three animals were selected from the lidocaine (6 mg)-treated, SRLS (20 mg)-treated, PLGA-only, sham-surgery (no drug administration), and normal rat groups. We also assessed histopathology of the SRLS-treated group 1 month after drug administration. The rats from each group were then killed with an intraperitoneal injection of sodium pentobarbital (100 mg/kg). The sciatic nerve and surrounding muscle were removed and fixed overnight in 0.1 M phosphate-buffered 4% paraformaldehyde. Serial dehydration with graded ethanol and xylene was followed by embedding in paraffin. The tissue was cut at 3-μm thickness and stained with hematoxylin and eosin. The slides were assigned an inflammatory reaction score (0 = normal, 1 = mild, 2 = moderate, and 3 = severe inflammatory reaction) by a neuropathologist unaware of the experimental group.

Statistics

Data were normally distributed and are shown as mean ± SD or SEM. Statistical analysis was conducted using Sigmapstat (Version 3.1; Systat Software, Inc., San Jose, CA). Behavioral studies were analyzed using two-way ANOVA followed by a Student–Newman–Keuls post hoc test. Immunohistochemical data were analyzed using one-way
ANOVA, followed by a Student–Newman–Keuls post hoc test. A P value less than 0.05 (one-tailed) was considered statistically significant.

Results

Production of Controlled Release Lidocaine and Characterization of Release Profile

We produced an SRLS containing 30% lidocaine using the PLGA polymer (polylactic acid:polyglycolic acid 50:50, molecular weight 85,000) that continuously released lidocaine for several days to 1 week. The in vitro release profile in phosphate-buffered solution is shown in figure 1A. The SRLS released approximately half of the lidocaine content within 72 h and 90% within 7 days in vitro but not in the rats. For residual SRLS removed from the administration site in rats, Data are shown as mean ± SD (n = 4).

Behavioral Study

Paw incision induced mechanical hypersensitivity as indicated by a reduced paw withdrawal threshold (fig. 2). Mechanical hypersensitivity was observed 2 h after paw incision when compared with presurgical values in the control group (administered PLGA only). Perineural administration of lidocaine produced antihypersensitivity effects when compared with the control group (P < 0.001, by two-way ANOVA). The paw withdrawal threshold in the lidocaine-treated group was increased 2 h after paw incision (P < 0.05). The antihypersensitivity, however, disappeared 5 h after paw incision, and the paw withdrawal thresholds were not different from those in the control group thereafter. The group treated perineurally with SRLS showed increased withdrawal thresholds when compared with other groups (P < 0.001, by two-way ANOVA). In the SRLS-treated group, the withdrawal thresholds were higher at all time points after paw incision when compared with the control group, and higher from 5 to 72 h after paw incision when compared with the lidocaine-treated group (P < 0.05; fig. 2). In rats with subcutaneous implantation of the SRLS into
the back, the paw withdrawal thresholds were not different from those of the control group.

In the lidocaine-treated group, the left hind paw of all rats showed severe motor weakness (score 2) at 2 h, and three rats showed partial motor weakness (score 1) at 5 h after perineural administration. Ten hours after administration, none of the rats exhibited motor weakness. In the SRLS-treated group, only one rat showed partial motor weakness 2 h after administration. None of the rats exhibited motor weakness at 5 and 10 h after the administration (fig. 3). Twenty-four hours after administration and thereafter, none of the rats showed motor weakness in either group (data not shown).

 Serum Lidocaine Concentration Study
In the lidocaine (6 mg)-treated group, the mean serum lidocaine concentration steadily decreased from 1.03 μg/ml at 2 h to 0.167 μg/ml at 10 h after perineural administration (fig. 4). In the SRLS (30%, 20 mg)-treated group, serum lidocaine was not detectable (<0.1 μg/ml; fig. 4) at any time point after perineural administration, except for 1 rat in which 0.2 μg/ml lidocaine was detected at 2 h.

 C-fos Immunoreactivity
C-fos protein immunoreactivity of the lumbar (L4–L5 segments) spinal cord ipsilateral to the side of the paw incision was detected mainly in the superficial laminae at 2 h and over a wide area at 48 h after paw incision (fig. 5). When lidocaine was administered around the sciatic nerve, the number of c-fos immunoreactive neurons in laminae I–II was lower than that in the control group at 2 h after paw incision (P < 0.05). At other time points, however, the number of c-fos immunoreactive neurons in the lidocaine-treated group did not differ from that in the control group. In the SRLS-treated group, the number of laminae I–II c-fos immunoreactive neurons was lower than that in the control group at 2,

![Fig. 4. Serum lidocaine concentration in the lidocaine-treated and slow-release lidocaine sheet (SRLS)-treated group. Data are shown as mean ± SD (n = 6 in each group).](image)

![Fig. 5. Representative images of c-fos immunoreactive neurons in the ipsilateral spinal cord after paw incision for polylactic-coglycolic acid sheet (20 mg without lidocaine; control), slow-release lidocaine sheet (SRLS; 30%, 20 mg), or lidocaine (6 mg) groups. Increased c-fos expression was observed in the superficial internal area at 2 h (A) and over a wide area at 48 h (D) after paw incision. In the SRLS-treated group, a small number of c-fos–positive neurons was observed at all time points (B, E). In the lidocaine-treated group, some c-fos expression was observed at 2 h (C), but a wider area of c-fos expression was observed at 48 h (F). Bilateral image of the spinal dorsal horn (G). Scale bar = 100 μm.](image)
5, and 48 h after paw incision and lower than that in the lidocaine-treated group at 5 and 48 h after paw incision ($P < 0.05$; fig. 6A).

Similarly, in laminae III–IV, the number of c-fos immunoreactive neurons in the lidocaine-treated group was lower than that in the control group at 2 h after paw incision ($P < 0.05$). Similar to the other layers, the number of c-fos immunoreactive neurons in the lidocaine-treated group did not differ from that in the control group at the other time points. In the SRLS-treated group, the number of c-fos immunoreactive neurons was lower than that in the control group at 5 and 48 h after paw incision and also lower than that in the lidocaine-treated group at 5 and 48 h ($P < 0.05$; fig. 6C).

At 7 days after the incision, there was no difference in the number of c-fos immunoreactive neurons in any layer among the groups.

**Neurotoxicity and Muscle Toxicity Study**

In the PLGA and SRLS groups, inflammation characteristic of a foreign body reaction was present around the site of drug administration, with slight inflammation in the neighboring muscle and connective tissues, but little to no inflammation was detected within the nerve (fig. 7, A–C). In the lidocaine (6 mg)-treated group, however, inflammation together with reactive fibrosis was moderately observed in the muscle and nerve near the administration site (fig. 7, D–F). The infiltrating cells were mainly macrophages, giant cells of foreign body type, lymphocytes, and plasma cells. The histopathologic findings are summarized in table 1. One month after SRLS administration, there was little inflammation of the nerve and muscle, and we could still observe the SRLS in the administration site. However, we could not detect the SRLS at 3 months after administration.

**Discussion**

The goal of the current study was to manage postoperative pain with a single treatment of an ultralong-acting local anesthetic. We were able to produce an SRLS that continuously released lidocaine for 1 week, and sciatic nerve block with the SRLS decreased mechanical hypersensitivity for 1 week in a rat model of postoperative pain. In contrast, lidocaine administered as a powder only decreased mechanical hypersensitivity for 2 h. In immunohistochemical studies, c-fos expression in the spinal cord dorsal horn was suppressed for a longer interval in the rats treated with the SRLS when compared with lidocaine powder. Lidocaine in the SRLS-treated rats was mostly undetectable in the serum. Further, the SRLS-treated group did not show motor weakness or neurotoxicity, whereas the rats treated with lidocaine powder showed severe motor weakness and moderate inflammation around the nerve.

Although there are several reports of controlled-release local anesthesia using bupivacaine,

![Fig. 6. Quantification of c-fos immunoreactive neurons 2, 5, 48 h, and 1 week after paw incision and treatment with polyactic-coglycolic acid sheets (20 mg without lidocaine; control), slow-release lidocaine sheets (SRLS; 30%, 20 mg), or lidocaine (6 mg). Numbers of c-fos immunoreactive neurons are shown as mean ± SEM (six sections in each rat, $n = 6$ for each group). (A) c-fos immunoreactive neurons in laminae I–II, (B) c-fos immunoreactive neurons in laminae III–IV, (C) c-fos immunoreactive neurons in laminae V–VI. * $P < 0.05$ compared with the control group. # $P < 0.05$ compared with the lidocaine-treated group.](image-url)
these studies, the concentration of controlled-release lidocaine ranged from 4% to 20% w/w. We generated several types of sheets with different lidocaine concentrations, different polylactic acid:polyglycolic acid ratios, and different molecular weights of PLGA. In the current study, we succeeded in producing lidocaine sheets with a 30% concentration of lidocaine, which is the highest concentration ever reported. We did not use other combinations of SRLS in the current study because the lidocaine release profile was inadequate. During the postoperative period, severe pain continues for 2–3 days and then gradually decreases over the first week after surgery. Therefore, we tried to generate sheets that would release half of the lidocaine content within 2–3 days and the remaining lidocaine within 1 week after surgery. A higher lidocaine concentration showed burst release in the early phase (data not shown) and was, therefore, not useful for application as controlled-release lidocaine. Consequently, we determined that the ideal combination for postoperative pain management was lidocaine (30%, w/w) and PLGA (polylactic acid:polyglycolic acid = 50:50, molecular weight 85,000). Although we tried to form microspheres with PLGA and lidocaine, we could not produce slow-releasing microspheres with a high lidocaine concentration. The dose of lidocaine contained by the SRLS (20 mg: lidocaine 6 mg) sufficient to inhibit hypersensitivity after paw incision was determined in our preliminary experiments. This amount of lidocaine contained in the SRLS suppressed mechanical hypersensitivity after paw incision without inducing motor weakness.

We used a rat model of postoperative pain produced by paw incision. This model demonstrates hypersensitivity continuing for several days and has characteristics similar to those of human postoperative pain. The nature of hypersensitivity after paw incision is unique and differs from that following inflammatory or peripheral nerve injury. For example, although spinal N-methyl-D-aspartate receptor antagonists attenuate hypersensitivity in most models of persistent pain, they are not effective for treating hypersensitivity after paw incision. In contrast, intrathecal administration of non-N-methyl-D-aspartate receptor antagonists is effective. Further, although descending facilitation from the rostral ventromedial medulla contributes to behavioral hypersensitivity in diverse animal models of inflammatory and neuropathic pain, this mechanism is not involved in hypersensitivity after incision. In addition, although dorsal horn neurons are sensitized after paw incision, plantar injection of a local anesthetic completely reverses the sensitization. These findings suggest that peripheral mechanisms strongly contribute to hypersensitivity after paw incision and that a long-acting peripheral nerve block is a promising method for reducing postoperative pain. In the current study, hind paw incision induced a robust increase in the sensitivity to mechanical stimuli in the control group (rats treated with PLGA only). The withdrawal threshold decreased 2 h after paw

### Table 1. Inflammatory Reaction Score with Nerve and Muscle in Each Group

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<th>Muscle</th>
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<td>SRLS (1 mo)</td>
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<td>0, 0, 1</td>
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Three animals were selected from the lidocaine (6 mg)-treated, slow-release lidocaine sheet (SRLS) (30%; 20 mg)-treated, polylactic-coglycolic acid (PLGA) only, sham-surgery (no drug administration), and normal rat groups. Score: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe inflammatory reaction.

Fig. 7. (A–C) Sciatic nerve, surrounding muscle, and connecting tissue stained with hematoxylin and eosin 1 week after administration of slow-release lidocaine sheet (SRLS) or (D–F) lidocaine. (B, C) High-magnification images of (A); (B) focuses on the muscle, and (C) focuses on the nerve. Signs of inflammation were slightly detected in the muscle (B), but little to no inflammation was detected in the nerve (C). (E, F) High-magnification images of (D); (E) focuses on the muscle, and (F) focuses on the nerve. The macrophages, lymphocytes, and plasma cells were moderately observed in the muscle and nerve. Atrophy of the muscle fibers can also be observed in (E). M = muscle; N = sciatic nerve. Scale bars = 500 μm.
incision, and this decrease was maintained for 48 h. The withdrawal threshold then gradually increased up to 1 week after surgery, as described previously. The withdrawal threshold was higher in the SRLS-treated group than in the control group at all time points measured. In contrast, the withdrawal threshold in the lidocaine-treated group was higher than that in the control group only at 2 h after paw incision. This finding indicates that the SRLS was effective for 1 week, consistent with the in vitro lidocaine release study.

Sensory inputs, particularly nociceptive signals, are reported to produce increased expression of the immediate early gene c-fos and its protein product Fox. Noxious stimuli are transmitted by Aδ and C primary afferent fibers to nociceptive neurons in the spinal cord, and c-fos–positive neurons are principally located in laminae I, II, V, and VI of the spinal dorsal horn. Previous studies demonstrated that c-fos–positive neurons are increased in the spinal dorsal horn neurons in a postoperative pain model. C-fos expression in a postoperative pain model is inhibited by analgesic treatments using local anesthetics, morphine, or ketamine. In the current study, the time course of c-fos expression closely matched the results of the behavioral experiments. The number of c-fos immunoreactive neurons in laminae I–II and III–IV was lower in the SRLS-treated group than in the control group at 2, 5, and 48 h after incision. The number of c-fos immunoreactive neurons in laminae I–II, III–IV, and V–VI was also lower in the SRLS-treated group than in the lidocaine-treated group at 5 and 48 h after incision.

Little lidocaine was detected in the serum of the SRLS-treated group. In contrast, even though an identical amount (6 mg) was administered, lidocaine was detected in the serum of the lidocaine-treated group for up to 10 h after administration. Previous studies showed that even at very low plasma levels (100–200 ng/ml), systemic lidocaine produced antihyperalgesic action in humans and animals. In the current study, we subcutaneously placed the SRLS into the backs of rats with paw incision and assessed antihyperalgesic action. Although we did not measure the serum lidocaine level after subcutaneous administration of SRLS, we could not detect any antihyperalgesic effect. This finding suggests that SRLS only acts at the applied site without systemic action.

The histopathologic findings demonstrated that placement of the SRLS near nerve and muscle tissues induced less inflammation when compared with lidocaine powder treatment. We could not detect neural toxicity during behavioral testing or routine histologic examination. More specialized axonal imaging with either high-powered light microscopy or electron microscopy may, however, detect evidence of axonal toxicity. Our results suggest that slow-release lidocaine has the advantage of not only producing long-term antihyperalgesic effects after paw incision but also preventing inflammation induced by high local concentrations of lidocaine. In the current study, we placed the SRLS directly around the sciatic nerve ipsilateral to the paw incision via surgical exposure of the nerve. When the surgical field is adjacent to the innervating nerve, direct in situ placement of the SRLS during surgery might be applicable. Clinically, however, nerve block or wound infiltration with local anesthetics via needle injection is more typically used for postoperative pain management. Because the lidocaine release profile for the SRLS generated in this study suggests a strategy for the management of postoperative pain, our future studies will produce novel lidocaine microspheres that can be injected via a needle.

In summary, we produced an SRLS and used it for sciatic nerve block in the rat model of postoperative pain. Single treatment of this controlled-release lidocaine produced an antihypersensitivity effect for 1 week without inducing inflammation of the sciatic nerve. C-fos expression in the spinal cord dorsal horn was consistent with the results of the behavioral experiments. This slow-release technique for administration of a local anesthetic might be a promising method for postoperative pain management, as physicians could avoid the risks of side effects produced by the systemic injection of opioids or continuous infusion of local anesthetics with an indwelling catheter.

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References
8. Mowat JJ, Mok MJ, MacLeod BA, Madden TD: Liposomal bupivacaine: Extended duration nerve blockade using large unilamellar vesicles that exhibit a proton gradient. ANESTHESIOLOGY 1996; 85:635–43.


33. Xiao WH, Bennett GJ: C-fiber spontaneous discharge evoked by chronic inflammation is suppressed by a long-term infusion of lidocaine yielding nanogram per milliliter plasma levels. Pain 2008; 137:218–28