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ORIGINAL ARTICLE

An injectable controlled-release local anesthetic formulation of levobupivacaine based on a temperature-responsive polymer: Evaluation of analgesia, motor impairment, and histological toxicity in rats

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Abstract

Purpose Postoperative pain management is extremely important for early recovery after surgery. However, effective and safe techniques for controlling postoperative pain are lacking. This study examined the effectiveness of controlled-release levobupivacaine for creating sciatic nerve blocks in a rat model of postoperative pain.

Methods A novel controlled-release injectable levobupivacaine gel was produced using a triblock copolymer of poly(ε-caprolactone-*co*-glycolide) and polyethylene glycol (tri-PCG). Male rats were used to create the incisional pain model. A single dose of controlled-release levobupivacaine (2.25%) gel, 0.25% levobupivacaine (clinical use), or tri-PCG was injected around the sciatic nerve of each rat immediately before paw incision. The pain thresholds were assessed preoperatively and up to 48 h postoperatively using von Frey filaments. Side effects were assessed using a motor impairment test, levobupivacaine blood level measurements, and pathological assessments.

Results The novel controlled-release levobupivacaine exhibited temperature-responsive sol–gel transition. In vitro, this formulation released 60% of its levobupivacaine content within 24 h. The withdrawal threshold was higher in the controlled-release levobupivacaine group than in the 0.25% levobupivacaine group at 6 and 12 h after paw incision. Motor impairment was not observed after controlled-release levobupivacaine injection, and the levobupivacaine blood level remained below the limit of detection throughout the assessment. On histopathology, weak signs of inflammation were detected in rat muscle and nerve tissues in the controlled-release levobupivacaine group.

Conclusion A single injection of controlled-release levobupivacaine gel almost safely inhibited hyperalgesia for 12 h in a rat model. However, further research is needed on its effects on the surrounding tissue.

Keywords Local anesthetic · Controlled-release · Temperature-responsive polymer · Rat model

Introduction

In recent years, the concept of enhanced recovery after surgery has gained popularity, and the need for adequate postoperative analgesia has been recommended in such programs [1, 2]. Analgesic methods include the systemic administration of opioids, acetaminophen, and non-steroidal anti-inflammatory drugs; local anesthesia around the wound; nerve blocks to inhibit pain perception in peripheral nerves; and epidural analgesia throughout the administration of local anesthetics [1–3].

Intravenous patient-controlled analgesia (IV-PCA) maximizes the efficacy of opioids while reducing their side

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effects. IV-PCA is an extremely useful method for preventing opioid blood levels from falling outside the target range. However, patients might be unable to use IV-PCA appropriately because of low consciousness or impaired judgement and cognition. In addition, IV-PCA cannot completely prevent the side effects of opioids themselves.

Various types of local anesthetics are currently used for pain relief, but their effects persist for only a few hours at most. Therefore, continuous catheter-based administration is used for prolonged analgesia. However, its use is limited by its risk of infection and its tendency to increase hospital stay and costs. The epidural administration of local anesthetics associated with nerve blocks has been re-evaluated as previously described. Epidural administration provides better analgesia than IV-PCA for both pain at rest and pain during physical activity. However, it has significant disadvantages, including risks of incorrect subarachnoid puncture, spinal cord and nerve root damage, and severe problems attributable to hematoma and infection.

When new local anesthetics are developed to extend the effective time, the prolonged duration of activity often results in increased toxicity [4]. Therefore, controlled-release local anesthetics using drug delivery systems (DDSs) are considered alternatives for extending the effective time [5, 6].

Local anesthesia using controlled-release bupivacaine has been studied since the 1990s, and it finally entered clinical use in 2011 following US Food and Drug Administration approval. Controlled-release bupivacaine is encapsulated in liposomes. Liposomes are gradually degraded in the body, resulting in the slow release of encapsulated bupivacaine. It has not been approved in Europe and is not available for clinical use in Japan. Meanwhile, levobupivacaine is a formulation containing only the S optimal isomer of bupivacaine. It is safer than bupivacaine because it is less cardiotoxic and neurotoxic, and it is less cardiotoxic than ropivacaine with similar analgesic potencies [7], [8].

The main characteristic of gel formulations is that they are temperature-responsive, existing as a liquid at room temperature and gelling around body temperature, permitting easy injection and allowing the formulations to gel and stay in place in the body. One typical example is an ABA-type triblock copolymer of poly(lactide-*co*-glycolide) and PEG, which is marketed as ReGel® [9]. ReGel® is characterized by a liquid state at room temperature (25 °C) and gelation around body temperature (37 °C). This means that the drug can be injected easily using a needle, and after administration, it changes to a gel form that remains at the site of administration, permitting sustained drug release. Sustained release is achieved by the gradual dissolution of levobupivacaine from the gelatinized base. Because ReGel® is biodegradable, and it does not accumulate in the body, it is promising from a DDS perspective. Sustained drug-release devices using such injectable polymer systems have been studied [9–11]. Because of the appropriate crystallinity of the hydrophobic segments, this polymer is a powdery solid after freeze-drying the aqueous solution, and it can be quickly dissolved in aqueous solution [12–14]. Some researchers previously reported this drug-release system and other biomedical applications using the polymer [14–18].

In this study, we developed a low-toxicity, long-acting local anesthetic formulation of levobupivacaine using a temperature-responsive, biodegradable polymer. We examined the release kinetics of the prepared sustained-release levobupivacaine formulation and evaluated its analgesic and adverse effects in a rat postoperative pain model.

Materials and methods

Controlled-release levobupivacaine formulation

Tri-PCG was synthesized via ring-opening polymerization of ε-caprolactone (CL) and glycolide using PEG (molecular weight [MW] = 1500 g/mol) as a macroinitiator according to a previously reported method [13, 14]. The total MW of tri-PCG was 5300 g/mol. The degrees of polymerization for CL and glycolic acid (GA) were 14 and 4.3, respectively. The molar ratio of CL/GA in the copolymer was 3.4. The controlled-release formulation was prepared by dissolving tri-PCG (25 wt%) in a 3.0 wt% levobupivacaine–saline mixture. Tri-PCG was dissolved by physical mixing followed by vortexing for at least 30 min. The solution was then boiled in hot water at approximately 90 °C, vortexed, and cooled. Then, the solution was subjected to at least three additional cycles of boiling and vortexing. The final formulation was then prepared by removing air bubbles created during stirring using ultrasound while cooling with water at 0 °C.

Sol–gel transition behavior evaluation

To identify the temperature at which tri-PCG enters a liquid or gelled state, these samples in test tubes were immersed in a water bath set at various temperatures for 10 min each. The tubes were inverted in the water bath for 30 s and evaluated by the test tube tilt method, in which the mixture was considered to be in solution if it flowed down and in a gelled state if no flow was observed (Fig. 1). Furthermore, the sol–gel transition behavior was evaluated using a dynamic rheometer (Thermo HAAKE RS600, Thermo Fisher Scientific, Waltham, MA, USA). We used this rheometer to determine at what temperature it changes from solution to gel and from gel to solution.

Rheometer is crucial for studying how materials flow and deform under stress or strain. Rheometer provides detailed insights into a material's viscoelastic properties and these

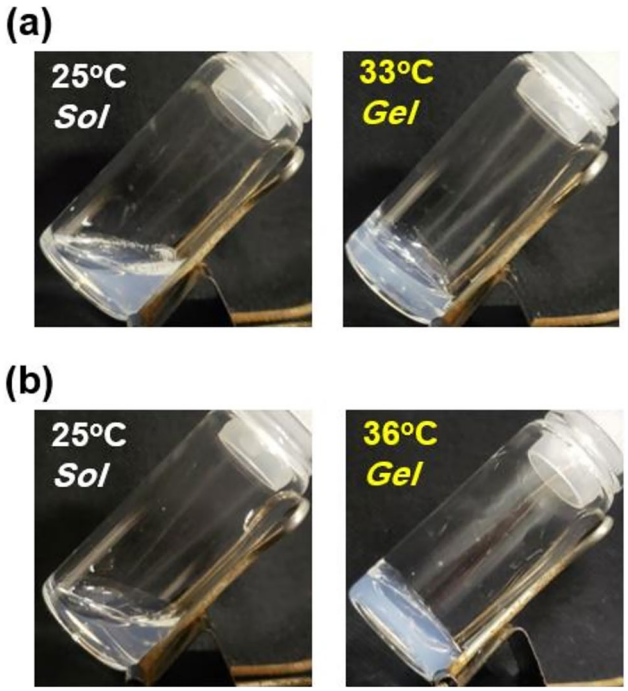

Fig. 1 Sol–gel transition behavior. Photographs of **a** tri-PCG and **b** levobupivacaine hydrochloride/tri-PCG solutions as a solution or gel. tri-PCG, triblock copolymer of poly(ε-caprolactone-*co*-glycolide) and polyethylene glycol

measurements are particularly important in industries like polymers and pharmaceuticals. The storage modulus (G') measures the elastic behavior of a material. Materials with a high G′ return to their original shape easily and resist deformation, such as solid rubber or gels. The loss modulus (G") measures the viscous behavior of a material. Materials with a high G″ dissipate energy and flow, such as liquids or soft polymers. Rheometers are used to test materials that change with temperature, such as thermoresponsive polymers. Rheometers are essential for understanding how materials respond to stress and temperature changes. The heating rate was set at 0.5 °C/min. The storage modulus (G′) and loss modulus (G″) of the formulations at 20–50 °C were observed, and the gelation temperature was defined as the crossover point of G′ and G″.

In vitro levobupivacaine release study

The capacity of the slow-release levobupivacaine gel (SRLBG) to release levobupivacaine in vitro was determined before the study. A solution of levobupivacaine hydrochloride and tri-PCG was gelled by incubation in a thermostatic bath at 37 °C for 10 min. Saline solution (30 mL, 37 °C) was gently added on the gels, followed by incubation at 37 °C. The supernatant (0.1 mL) was collected at predetermined intervals, and a new saline solution (0.1 mL) was added. The amount of levobupivacaine hydrochloride present in the collected supernatant was determined by high-performance liquid chromatography (HPLC). The HPLC system (GPC-8020 series system, JASCO) was equipped with a C18 column (particle size, 3.5 µm, 4.6 × 150 mm), and the temperature was maintained at 40 °C. The mobile phase consisted of a mixture of acetonitrile (20 mM, pH 8.0) and sodium phosphate buffer (50:50, v/v), and the flow rate was set to 1.0 mL/min. Detection was performed at 220 nm using a UV detector. Calibration standards of levobupivacaine were prepared at concentrations of 0.1–250 µg/mL, and a standard calibration curve was established by plotting the peak areas against the known concentrations. The values were expressed as the mean ± standard deviation (SD) of three replicates.

Animals

This investigation was approved by the Animal Care and Use Committee of Gunma University Graduate School of Medicine (Maebashi, Japan: approval number: 23–003). In total, 105 male Sprague–Dawley rats weighing 250–300 g were used in all experiments. Rats were housed in an environment with free access to water and food under a 12-h/12-h light/dark cycle. Data and specimens were collected from all rats. Rats were euthanized when data collection was completed or when pathology specimens were prepared. Euthanasia was performed using a decapitation device under deep anesthesia with isoflurane.

Drug application

Under isoflurane anesthesia (2% isoflurane in 100% oxygen), 0.2 mL of the preparation were injected around the left sciatic nerve using a landmark technique as described by Thalhammer et al. [19]. The rats were placed in the side-lying position with the left hind limb at a right angle to the longitudinal axis of the trunk. The positions of the greater trochanter and sciatic tubercle were confirmed by palpation. On an imaginary line from the greater trochanter to the sciatic tubercle positioned approximately one-third caudally from the greater trochanter, the 26G needle was advanced dorsolaterally at a 45° angle until the tip contacted the sciatic bone, and the drug was administered. Four groups were used (eight rats per group): 2.25% SRLBG, 0.25% levobupivacaine (on clinical use), tri-PCG alone, and drug-free groups. The person performing the behavioral tests was blinded to the drug administration. Groups were distinguished by marking the rats' tails, and matching was done by a researcher not involved in animal testing.

Pain model

The postoperative pain model was generated as described by Brennan et al. [20]. In this model, the pain caused by an incision is profound and persistent. It is characterized by reduced withdrawal thresholds, suggesting that mechanical hyperalgesia is present, and this results in behaviors that are timed similarly to pain measures in postoperative patients. After drug injection, a 1-cm incision was made immediately distal to the heel on the plantar surface of the left hindfoot under continued isoflurane anesthesia. The plantaris muscle was then lifted with forceps and debrided. Finally, the wound was closed with mattress sutures using 5–0 silk thread. After surgery, the rats were allowed to recover from anesthesia in the cage. The wound was checked to ensure that there were no major problems before behavioral experiments were performed.

Behavioral testing

von Frey Test (mechanical stimulus escape threshold test)

Behavioral testing was conducted in accordance with the method described by Tobe et al. [21]. Mechanical hyperalgesia is critical property of incisional pain. It was detected as a decreased pain threshold and an increase in the pain response to suprathreshold stimuli. Rats were placed in individual plastic chambers with a plastic mesh floor and allowed to acclimate to the environment for 15 min. The mechanical withdrawal threshold was determined using calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA). These filaments are nylon monofilaments with similar lengths and varying diameters. As the diameter of the filament increases, the force necessary to bend it increases. 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 by Chaplan et al. [22]. Assessments were conducted before and 1, 2, 6, 12, 24 and 48 h after drug administration. Four groups were used (eight rats per group): 2.25% SRLBG, 0.25% levobupivacaine (on clinical use), tri-PCG alone, and drug-free groups. This behavioral study was performed in a randomized, blinded manner.

Motor impairment test (assessment of side effects)

When the local anesthetic concentration is excessive, the agent acts on the motor nerves, manifesting as symptomatic paralysis. In addition, prolonged exposure to high concentrations of local anesthetics can have long-term adverse effects. Motor weakness in the affected paw was evaluated using the motor impairment score as described by Gianolio et al. [23] as follows: 0 (normal), the rats were able to walk and grasp normally; 1 (partial blockade), when walking, the rats walked, gathered the forepart of the foot, kept it sideways, and exhibited limited ability to grasp the bars; and 2 (severe blockade), the rats dragged their legs and failed to grasp the bars upon elevation of their hind limbs. The assessment was performed before and 1, 2, and 6 h after drug administration. The administered drugs were tri-PCG, 2.25% SRLBG, 2.25% levobupivacaine, and 0.25% levobupivacaine (on clinical use). Eight rats were used in each group. This motor impairment assessment was performed in a randomized, blinded manner.

Blood concentration measurement

We measured blood levels over time in rats treated with SRLBG and 0.25% levobupivacaine to evaluate toxicity. Three samples each were collected at 1, 2, 6, 12, 24, 36, 48 and 60 h after administration in the SRLBG group and at 1, 2 and 6 h after administration in the 0.25% bupivacaine group. The blood samples were centrifuged, the serum was stored, and the levobupivacaine concentration was determined by HPLC at Kansai University. The HPLC system setting was the same as that in the release study. Detection was performed at 220 nm using a UV detector. Calibration standards of levobupivacaine were prepared at concentrations of 0.1–250 μg/mL, and a standard calibration curve was established by plotting the peak areas against the known concentrations.

Histopathology

We examined the histopathology of the muscles and nerves surrounding the site of administration. In the histopathological evaluation, SRLBG, tri-PCG, 0.25% levobupivacaine, and 2.25% levobupivacaine were administered in a volume of 0.2 mL around the sciatic nerve to two animals each using the aforementioned landmark method [20]. Tri-PCG is the base material of SRLBG, and 2.25% levobupivacaine is a considerably higher concentration than used clinically. In each group, the thigh tissue around the left sciatic nerve was removed from the two animals at 48 h and 2 weeks after drug administration, respectively. Each tissue sample was fixed in formaldehyde, and a paraffin block was prepared. Sections cut from the blocks were stained with hematoxylin–eosin, and changes in histological findings were observed. Assessment and diagnosis were performed by a pathologist unaware of experimental group assignment. In this experiment, two rats were used for each drug group in a two-time course, and thus, 16 rats were used.

We counted the number of infiltrating inflammatory cells in pathological specimens in which each drug was administered by two pathologists to the sciatic nerve and compared the findings between the group that received tri-PCG (tri-PCG and SRLBG) and the group that did not receive tri-PCG (0.25% levobupivacaine and 2.25% levobupivacaine).

Statistical analysis

The sample size was calculated using a power and sample size calculator. To detect a 92% difference at 12 h between the SRLBG and 0.25% levobupivacaine groups (estimated from our pilot observations) with 80% power and a 5% alpha error, a sample size of eight rats per group was required. The results obtained from the von Frey experiments were compared by the Holm–Sidak method. Inflammatory cells' count was analyzed normality using Shapiro–Wilk test. If a normal distribution was confirmed, a Student's *t*-test was performed; otherwise, a Mann–Whitney U test was performed. All analysis was performed using HULINKS Sigma Plot 14.5. The significance level was set at 0.05.

Results

Evaluation of sol–gel transition behavior

The results of the test tube tilt method are presented in Fig. 1. The mixture was flowed down at 25℃, indicating that it was in solution, whereas no flow as observed at 33 and 36℃, indicating a gelled state. As presented in Fig. 1a), tri-PCG, which was used to make the sustained-release drug in this study, was in a liquid state at 25 °C and in a gelled state at 33 °C. As highlighted in Fig. 1b), controlled-release levobupivacaine was also in a liquid state at 25 °C but in a gelled state at 36 °C.

Rheological measurements

Rheological measurements revealed that the temperature at which G′ exceeded G″ for the levobupivacaine hydrochloride-tri-PCG mixture was 30.8 °C. The temperature at which G′ became smaller than G″ was 54.7 °C (Fig. 2). These results, which indicated that the drug gels at 37 °C, suggesting that it could be used as a controlled-release drug that gels in vivo.

In vitro levobupivacaine release study

The calibration curve for levobupivacaine was linear, with a correlation coefficient (r^2) of 0.998. The cumulative release of levobupivacaine from SRLBG into saline solution was calculated (Fig. 3). The mean release rates of levobupivacaine from SRLBG at 1, 2, 6, 12, 24, 36, 48, 60, and 72 h were 15.1% ± 0.1%, 20.6% ± 3.9%, 36.1% ± 2.3%, 48.3% ± 3.2%, 62.8% ± 1.9%, 68.4% ± 2.4%, 71.2% ± 1.8%, 71.0% ± 1.8%, and 70.8% ± 1.7%, respectively. Nearly 50% of the loaded levobupivacaine was released within 12 h, whereas more than 60% was released within 24 h. The release rate was somewhat rapid, but a certain level of controlled release was achieved.

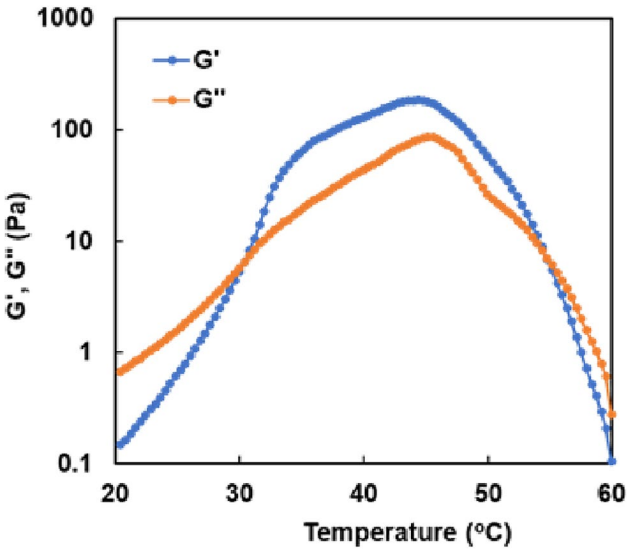

Fig. 2 Rheological measurements. G′ and G″ of levobupivacaine hydrochloride/tri-PCG solution as a function of temperature. The rate of the temperature increase was 0.5℃/min. G′, storage modulus; G″, loss modulus; tri-PCG, triblock copolymer of poly(ε-caprolactone-*co*-glycolide) and polyethylene glycol

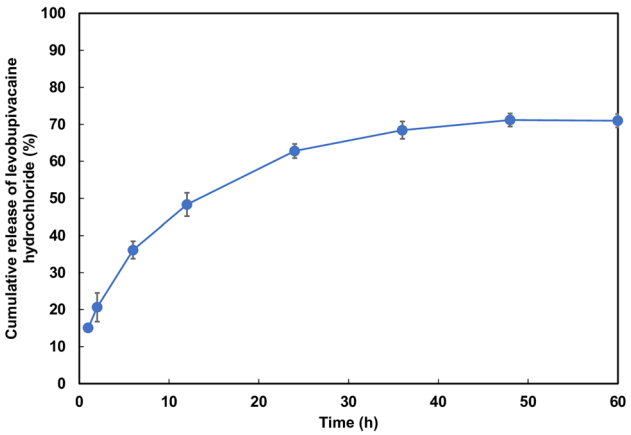

Fig. 3 Results of the in vitro levobupivacaine release study (n = 3, mean ± SD)

Behavioral testing

von Frey Test

The results of the pain threshold assessment test are presented in Fig. 4.

In a previous study by Tobe et al. [21], the withdrawal threshold in a rat model of postoperative pain was 5–10 g at 2–48 h after surgery. There was no significant difference in the withdrawal threshold in the drug-free (sham) group in this study. The paw withdrawal thresholds were significantly higher in the SRLBG group than in the 0.25% levobupivacaine group at 6 and 12 h after administration. At 24 h, the threshold tended to be slightly high in the SRLBG group, but the difference was not significant. The threshold did not significantly differ between the tri-PCG and sham groups. Meanwhile, the pain threshold was significantly higher in the 0.25% levobupivacaine group than in the tri-PCG group at 2 h after injection, but the difference was not significant after 6 h.

Motor impairment study

The results of the motor impairment analysis are presented in Fig. 5. These results include the scores of all rats. All rats in the tri-PCG group exhibited no paralysis in all time courses. One hour after administration, one rat in the SRLBG group had severe motor impairment, and two rats had partial motor impairment. Conversely, only one rat had partial motor impairment in the 0.25% levobupivacaine group, whereas the other seven rats were normal. However, no animals exhibited motor impairment after 2 h in 0.25% levobupivacaine and SRLBG groups. Compared with the findings in the 2.25% levobupivacaine group, the motor impairment score was lower in the SRLBG group after 1 and 2 h. No rats had motor impairment in each group at 6 h after administration.

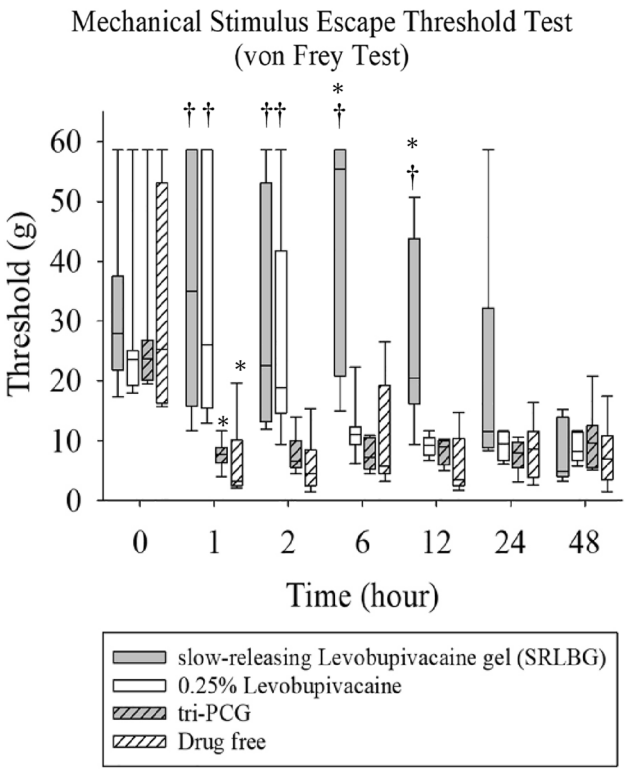

Fig. 4 Results of the mechanical stimulus escape threshold test (n = 8 in each group, median and interquartile ranges). *P < 0.05 vs. 0.25% levobupivacaine, †P < 0.05 vs. sham by the Holm–Sidak test

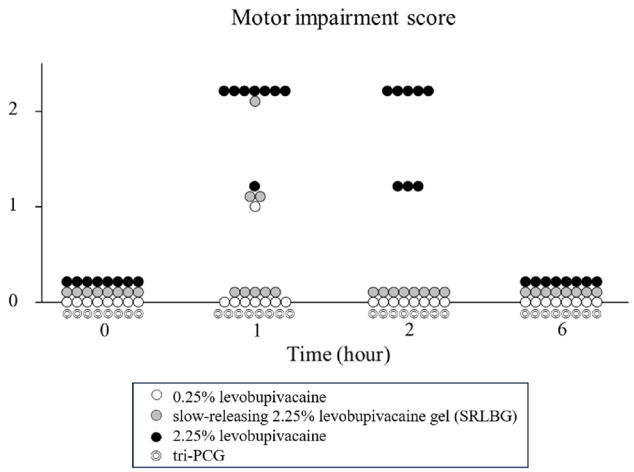

Fig. 5 Motor impairment score in the groups. 0; normal, 1; partial blockade, 2; severe blockade (n = 8 in each group). tri-PCG, triblock copolymer of poly(ε-caprolactone-*co*-glycolide) and polyethylene glycol

Blood concentration measurement

The concentration of levobupivacaine in each blood sample was determined by HPLC at Kansai University. Calibration curve was created from the peak area at a retention time of 10.8 min at 220 nm. The calibration curve for levobupivacaine was linear, with r^2 of 0.997. All samples had a levobupivacaine concentration lower than 0.5 µg/mL.

Histopathology

Photographs of histopathological specimens taken 48 h and 2 weeks after drug administration are presented in Fig. 6. There was little change in the pathology assessment at 1 and 2 weeks; thus, the results at 48 h and 2 weeks after administration are presented.

At 48 h after administration, inflammatory cell infiltration was observed at the site of drug administration in each group. Inflammatory cell infiltration was observed after 2 weeks in the SRLBG and tri-PCG groups but not in the

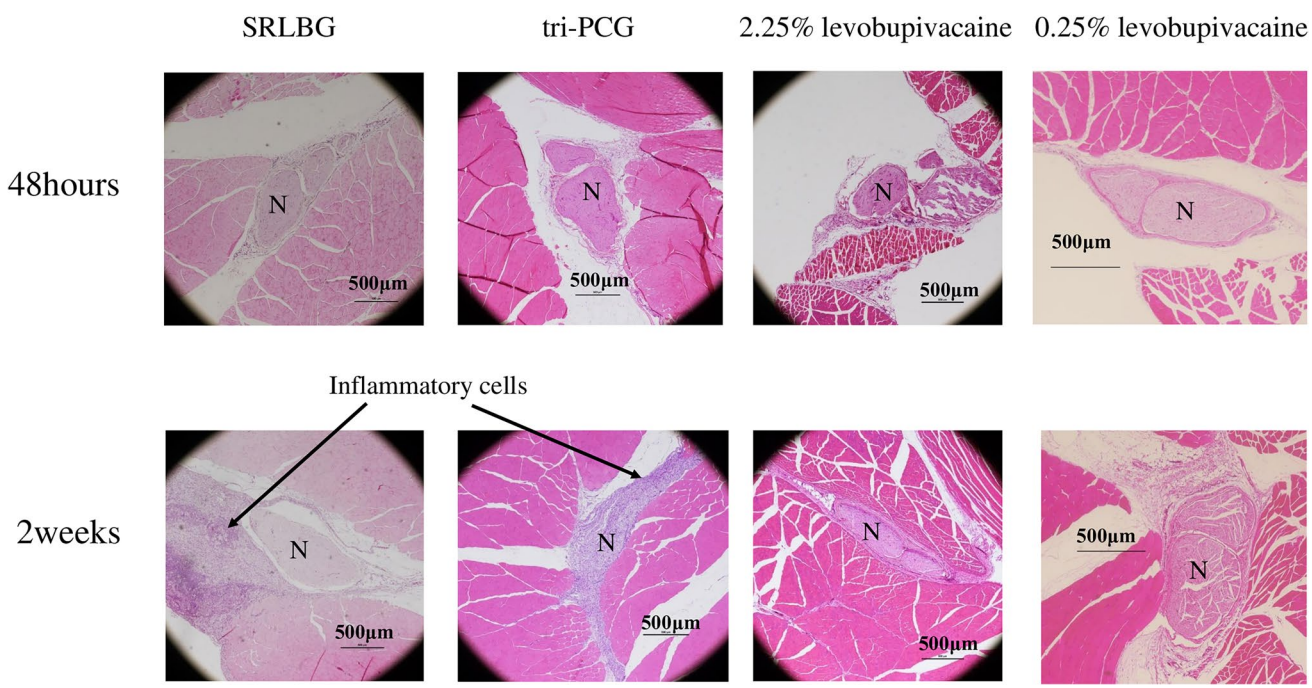

Fig. 6 Histopathological assessment at the site of drug administration at 48 h and 2 weeks after injection in each group (two rats in each group at each time point). tri-PCG, triblock copolymer of poly(ε-caprolactone-*co*-glycolide) and polyethylene glycol; SRLBG, slow-release levobupivacaine gel; N, sciatic nerve

0.25% and 2.25% levobupivacaine groups. Inflammatory cell infiltration was observed, but the effects on nerves and surrounding muscle tissue were not severe.

The number of infiltrating inflammatory cells was counted in pathology specimens after each drug was administered around the sciatic nerve by two pathologists after 48 h and 2 weeks using four specimens in the group administered tri-PCG (tri-PCG, SRLBG) and four specimens in the group that did not receive tri-PCG (0.25% and 2.25% levobupivacaine). The number of inflammatory cells counted is presented in Fig. 7. Clearly, the number of inflammatory cells was higher in the group administered tri-PCG, and inflammatory cell infiltration appeared to continue for more than 2 weeks. Almost all inflammatory cells were foamy histiocytes. Using the Shapiro–Wilk test, the results had a normal distribution at 48 h but not at 2 weeks. The results at 48 h were analyzed using Student's *t*-test, which revealed no significant difference between the two groups (mean ± SD: 1322.5 ± 1391.97 vs. 513.37 ± 578.32, P = 0.343). The results at 2 weeks were analyzed using Mann–Whitney U test, and the results revealed that the median number of inflammatory cells was significantly higher in the tri-PCG–administered group (median [interquartile range]: 6015.5 [3403.0–23695] vs. 138.5 [93.1–219.9], P = 0.029).

Fig. 7 Inflammation cell counts. Two pathologists each counted the number of inflammatory cells infiltrating around the sciatic nerve in the tri-PCG group (SRLBG + tri-PCG) and non tri-PCG group (0.25% levobupivacaine and 0.25% levobupivacaine). tri-PCG, triblock copolymer of poly(ε-caprolactone-*co*-glycolide) and polyethylene glycol; SRLBG, slow-release levobupivacaine gel

	tri-PCG group			Non tri-PCG group	
	Pathorogist A	Pathorogist B		Pathorogist A	Pathorogist B
48h-1	104	121	48h-5	172	196
48h-2	293	283	48h-6	197	183
48h-3	1778	1879	48h-7	320	285
48h-4	3117	3005	48h-8	1322	1432
2w-1	2579	2672	2w-5	99	85
2w-2	5639	5832	2w-6	101	92
2w-3	6135	6456	2w-7	188	173
2w-4	28963	30023	2w-8	245	221

Discussion

The novel controlled-release levobupivacaine exhibited temperature-responsive sol–gel transition. In vitro, this formulation released 50% of its levobupivacaine content within 12 h and 60% within 24 h. The withdrawal threshold was higher in the controlled-release levobupivacaine group than in the 0.25% levobupivacaine group at 6 and 12 h after paw incision. Motor impairment was not observed after controlled-release levobupivacaine injection, and the levobupivacaine blood level remained below the limit of detection throughout the assessment. On histopathology, the number of inflammatory cells was higher in the group administered tri-PCG and controlled-release levobupivacaine, and inflammatory cell infiltration appeared to continue for more than 2 weeks.

This study attempted to prolong the duration of action of levobupivacaine, a long-acting local anesthetic, by creating a controlled-release formulation. In addition, tri-PCG was chosen as a modality for producing the injectable controlled-release formulation. As indicated by the results, some success was achieved in creating an injectable controlled-release formulation. Gelation was observed at 36 °C, and thus, the agent is expected to remain at the site of administration after injection. Controlled-release levobupivacaine is expected to exhibit fewer side effects such as cardiotoxicity and central neuropathy than controlled-release bupivacaine formulations currently in clinical use. In addition, because it is injectable, the developed formulation can be used in ultrasound-guided peripheral nerve blocks, the value of which has been reassessed in recent years and the use of which has increased, illustrating that this treatment could be suitable for a wide range of situations. If the safety of the formulation can be confirmed, it might be possible to administer it into the epidural space or nearby peripheral nerves. Compared with continuous drug administration via indwelling catheters, which is currently used to prolong duration, the developed approach is expected to offer various advantages, including a reduced risk of infection, no accidental withdrawal of the catheter because of patient movement, and improved patient quality of life because of elimination of the need for an uncomfortable catheter.

In the in vitro release study, nearly half of the cumulative levobupivacaine content was released from SRLBG within 12 h. Levobupivacaine is released from SRLBG via three processes. The first is release from the gel by simple diffusion. Second, the gel dissociates, and the drug is released. Third, the polymers comprising the gel are hydrolyzed, causing the gel to collapse and leading to drug release. In this study, the release was relatively fast, and thus, it was most likely that the release was attributable to simple diffusion. Because the duration of action of 0.25% levobupivacaine in clinical use is approximately 3 h and SRLBG releases approximately half of its content in 12 h, it can be assumed that 0.25% levobupivacaine was administered continuously.

In vivo behavioral testing also demonstrated that, as previously mentioned, controlled-release levobupivacaine significantly increased the pain threshold at 6 and 12 h compared with the effect of 0.25% levobupivacaine. Although the high escape threshold is partly due to uncertainty as to whether it is sensory loss or motor paralysis, as shown in Fig. 5, there was only a slight motor paralysis in the 0.25% levobupivacaine and SRLBG groups at 1 h after administration. Therefore, it seems likely that this is basically a sensory paralysis, or in other words, an analgesic effect. This result was attributable to successful prolongation of the duration of action of the drug, and the effect was expected to be similar to that in the in vitro study. Paw withdrawal thresholds tended to be higher in the SRLBG group than in the other groups even after 24 h, although no statistically significant differences were observed.

Concerning toxicity, no increase in blood levobupivacaine levels was observed after administration of the controlled-release formulation. This suggests that levobupivacaine is slowly released from the controlled-release formulation in the rat body. There were no extreme differences regarding motor impairment compared with the findings for 0.25% levobupivacaine, which is typically used clinically. In addition, compared with the effect of 2.25% levobupivacaine, the degree of motor impairment was milder for controlled-release levobupivacaine. This result was consistent with the in vitro release characteristics.

In the pathological evaluation, the treatment was compared with higher concentrations, as it could be imagined that clinically used concentrations would not cause problems. Forty-eight hours after administration, inflammatory cell infiltration was observed in all groups. Inflammatory cell infiltration remained detectable in the SRLBG and tri-PCG groups 2 weeks after administration. This was likely attributable to a prolonged reaction to the foreign substance. The number of infiltrating inflammatory cells appeared to differ markedly depending on whether tri-PCG was included. Therefore, a comparative study was performed. Although there were individual differences because of manner in which the sections were cut, significantly more inflammatory cells infiltrated in the tri-PCG–administered group. These long-term inflammatory cell infiltrates suggested a response to the base material. Histopathological evaluation revealed some inflammatory cell infiltration around the site of administration of controlled-release levobupivacaine, but according to the pathologist, the nerves and surrounding muscle tissue were minimally affected. For clinical use, we should conduct

further studies in larger animals with increased numbers of animals.

The present study has some limitations. One major limitation of this study is the potential species difference between the animal model and humans, which may limit the direct applicability of our findings to human physiology or pathology. The animal model used in this study does not fully replicate the complexity of the human pain, which is a significant limitation in translating these findings to clinical setting. And the relatively short observation period in this study may not fully capture long-term effects or chronic changes associated with the intervention.

Based on the results of this research, it appears that the novel controlled-release levobupivacaine formulation can be used with few safety concerns. However, further evaluation using large animals, including assessments of stochastic effects, might be necessary.

Conclusion

A single injection of a novel controlled-release levobupivacaine formulation inhibited hyperalgesia for 12 h in a rat model of postoperative pain with few adverse effects. However, further research is needed on the effects on the surrounding tissue. Controlled-release local anesthetics are promising for the management of postoperative pain based on their greater safety and efficacy.

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