Randomized Trial

The Effect of Epidural Resiniferatoxin in the Neuropathic Pain Rat Model

Mi Geum Lee, MD, PhD[1], Billy K. Huh, MD, PhD[2], Sang Sik Choi, MD, PhD[3],
Dong Kyu Lee, MD, PhD[3], Byung Gun Lim, MD,PhD[3], and Mi Kyoung Lee, MD, PhD[3]

From: 1Department of Anesthesiology and Pain Medicine, Gachon University Dongincheon Gil Hospital, Incheon, Korea.; [2]Department of Anesthesiology, Division of Pain Management, Duke University Medical Center, Durham, NC; [3]Korea University Guro Hospital, Seoul, Korea.

Dr. Mi Geum Lee is with the Department of Anesthesiology and Pain Medicine, Gachon University Dongincheon Gil Hospital, Incheon, Korea. Dr. Huh is with the Department of Anesthesiology, Division of Pain Management, Duke University Medical Center, Durham, NC 27710. . Dr. Choi is with the Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital, Seoul, Korea. Dr. Dong Kyu Lee is with the Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital, Seoul, Korea. Dr. Lim is with the Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital, Seoul, Korea. Dr. Mi Kyoung Lee is with the Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital, Seoul, Korea.

Address correspondence:
Mi Kyoung Lee, MD, PhD
Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital, Seoul, Korea.
E-mail: mknim@hotmail.com

Background: Resiniferatoxin (RTX) is a potent synthetic agonist for transient receptor potential vanilloid subtype 1 (TRPV1), which has a selectivity for antinociception. The analgesic effect of epidural RTX in a rat model of neuropathic pain has not yet been studied.

Objectives: The purpose of this study was to evaluate the analgesic effect of epidural RTX on neuropathic pain in a rat model to mechanical and thermal stimulation. The dose-related behavior changes and side effects were also studied.

Study design: A randomized, experimental trial.

Setting: Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital

Methods: A spinal nerve ligation model was prepared using male Sprague-Dawley rats (7 weeks old, weight 230-250 g). An epidural catheter was placed at the L4-L5 level. Each study group (n = 6) received a different dose of RTX: 100 ng, 500 ng, 1 µg, 2 µg, 4 µg and 10 µg. All substances were administered in 20 µL volume doses. The control group (n = 6) received 20 µL of normal saline. We evaluated the response to mechanical and thermal stimuli as well as the sedation score at both short-term (3 hours) and long-term (20 days) after the epidural RTX injection.

Results: Prolonged analgesia to thermal stimulation was preceded by a transient dose-dependent hyperalgesia (500 ng, 1 µg) or sedation (≥ 2 µg) during the initial 60 minutes after RTX administration. Marked sedation and hyperventilation were noted at higher doses (≥ 2 µg), while 2 out of 6 rats died with a 10 µg dose. ED50 for epidural RTX was 265 ng (95% confidence interval 216.1–324.9 ng). The increased latency to thermal stimulation continued for 20 days at RTX ≥ 1 µg. But the threshold to mechanical stimulation increased only in the acute period and returned to the baseline after 3-5 days, regardless of the administered dose.

Limitations: A histological examination by electron-microscopic staining was not performed. The observation period was not very long (20 days).

Conclusion: RTX has potential to be used in an epidural route for neuropathic pain in a rat model with a relatively small amount, which produces transitory improvement of mechanical hypersensitivity and prolonged thermal analgesic response.

Key words: Epidural administration, mechanical allodynia, mechanical hypersensitivity, resiniferatoxin, sedation, spinal nerve ligation rat model, thermal hyperalgesia.

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Resiniferatoxin (RTX), isolated from the latex of the cactus Euphorbium resinifera, is one of the most potent agonists of the transient receptor potential vanilloid subtype 1 (TRPV1) (1). The TRPV1 receptor is a ligand-gated, nonselective cation channel expressed predominantly on the peripheral and central terminals of small-diameter sensory neurons, including nonmyelinated peptidergic C-fiber neurons and lightly myelinated low threshold Aδ-fiber neurons (1). A previous report, which had sensitized TRPV1 knock-out mice, concluded that the TRPV1 receptor has a critical role for inflammatory sensitization to noxious thermal stimulation (2).

RTX is highly selective for sensory nerve terminals expressing TRPV1 receptors without affecting proprioception and motor function (3,4). In many animal studies RTX has been found useful for inflammatory pain, postoperative incisional pain, and bone cancer pain (1,3,5,6). It has been administered by variable routes: systemic by subcutaneous, intrathecal, epidural, intraganglionic, perineural, and directly to peripheral nerve endings (1,3-8).

RTX (100-300 μg/kg) application by subcutaneous systemic route can produce long-lasting analgesia (7,9), but such high doses yield nonlocalized, spatially disseminated effects associated with systemic desensitization of TRPV1 receptors (3). Intrathecal RTX has advantages in selective targeting and permanent deletion of the TRPV1 receptors with long-lasting analgesic effect (1,4,5). However, the usefulness of intrathecal RTX is limited to the lumbar area (1,5), and the intrathecal route has not been used for non-cancer pain in general pain clinics. Rather, it usually has been used for anesthesia or for the advanced metastatic cancer pain of terminally ill patients (8). Perineural RTX also can provide long-lasting localized pain control in comparatively low doses without major systemic reactions. But this route seems not to be suitable for severe pain secondary to a specific disease, which has characteristics generally more diffuse and are not localized to one or 2 dermatomes (4,6).

The epidural administration of analgesics has provided analgesia in a wide range of chronic pain. It has the advantage of selective targeting of spinal segments at any spinal level. It can reduce the total dose of the administered drug and has a low systemic uptake while achieving regional control (7). It is already known that epidural RTX shows enhanced effectiveness relative to systemic RTX in normal rats (7). But the therapeutic effect of epidural RTX has not yet been investigated in a spinal nerve ligation (SNL) rat model.

The objective of this experimental study was to evaluate the analgesic effect and safety of lumbar epidural RTX injections in an SNL rat model.

METHODS

Production of a Rat Model of Neuropathic Pain

The experimental procedure was approved by our institution's Animal Committee. Rats were housed in clear plastic cages with sawdust bedding at standard room temperature (20-22°C) and humidity (55-60%), under a 12-hour light/dark cycle.

The animals were acclimatized for at least 3 days and handled by a researcher prior to surgery. Male Sprague-Dawley rats (7 weeks old, weight 230-250 g), purchased from Orient Bio (Gyeonggi-do, Korea) were used. Anesthesia was induced with 5% isoflurane in O_2 at 2 L/min and maintained with 2% isoflurane/O_2 via a loose-fitting mask.

In our study, the SNL model was made by a cutting method instead of ligation (10). A 3-cm paramedian incision was made at the left L4–sacral area and the paraspinal muscle was removed to visualize the left L6 transverse process. Using small scissors, we removed the muscle completely and exposed the L4 and L5 spinal nerves. After separating the L4 spinal nerve, the L5 spinal nerve was cut and spread laterally. The fascia and skin were closed using sutures, and the animals were allowed to recover for 10 days prior to testing.

Rats that exhibited tactile hypersensitivity with a von Frey filament were enrolled, while those with motor deficiency or a failure to exhibit subsequent tactile hypersensitivity were excluded from further testing.

Epidural Catheterization

The enrolled rats were anesthetized using the same technique as above. After sterile preparation, a 3-cm midline incision was made at the L1-L2 level. The superficial fascia and muscles were dissected, and the L1 and L2 spinous processes were exposed. Holding the L2 spinous process with tooth forceps, we removed the L1 spinous process. The surrounding muscles were carefully separated until the ligamentum flavum was exposed. Using a blunted 26-gauge needle, a small hole was made at the center of the ligament flavum, and a PE-10 catheter (Becton Dickinson and Company, Franklin Lakes, NJ, USA) was advanced approximately 3-cm caudad until the tip reached the L4-L5 interspace. Poly-

ethylene glue (A cyanoacrylate; Aron-Alpha, Toagosei, Japan) was applied around the catheter entry site to prevent drug leakage. The catheter was tunneled subcutaneously near the neck and injected with 0.1 mL of 2% lidocaine as a test dose.

Cases where the test solution accidentally injected intrathecally or intravenously leading to sudden death were not included in the experiment (11). After confirming correct epidural catheter placement, the fascia and skin were sutured.

If the rats showed abnormal findings, such as limping gait or spinal deformity during the 2-day observation period, they were excluded from this study. The total tube length was 17 cm and the dead space was 12 μL.

Drugs and Epidural Administration

RTX (Sigma-Aldrich, St. Louis, MO) 1 mg was dissolved in 95% ethanol 1 mL and diluted using 0.9% normal saline to a concentration of 1 μg/μL and stored at -20°C.

Forty-two catheterized rats (weight 280-330 g at that period) were prepared. RTX was administered 2 days after the catheterization (12). The rats were randomly divided into 7 groups according to RTX concentration: control group (normal saline), RTX 100 ng, 500 ng, 1 μg, 2 μg, 4 μg and 10 μg groups (n = 6 per group). The injection volume was 20 μL for each concentration.

After anesthesia induction with 2% isoflurane/O2, RTX was injected slowly for 15-20 seconds via an epidural catheter using a microinjector syringe (Hamilton Company, Reno, Nevada, USA).

Behavioral Tests

Mechanical stimulation and the thermal test were performed by one researcher who was blinded to the drug. The responses to a graded mechanical stimulus with von Frey filaments (Stoelting, WoodDale, IL, USA) were measured. The rat was placed under a transparent plastic dome on a metal mesh floor for 20 minutes. A von Frey filament was applied to the mid-plantar surface of the left hind paw for 2-3 seconds. The 50% withdrawal threshold was determined using the up-down method (13) starting with a 2.0 g (19.608 milliNewton) filament. A rapid foot lift upon filament application was regarded as a withdrawal response. Interpolation of the 50% threshold was carried out according to the Dixon method (14).

Behavioral tests were performed before the SNL (pre), prior to the epidural drug administration (post), and 15-minute intervals for the first 180 minutes and then at 1, 3, 5, 7, 10, 15, and 20 days after RTX injection. Behavioral changes and the sedation score were documented for the first 180 minutes.

To assess thermal pain sensitivity, a modified Hargreaves' test (15) was conducted using a plantar test device (7371, Ugo Basile, Camerio, Italy). The animals were allowed to move freely on a glass floor within an open-top transparent plastic box for 20 minutes. A mobile radiant heat source (I.R. intensity: 60) was applied through the glass platform to the plantar surface of the left hind paws (16). To prevent thermal injury, the exposure cutoff time was 15 seconds (16). Three consecutive measurements were averaged. The surface of the glass was maintained at 28-30°C using a heating pad and electric stove (10). Visible tissue damage was not observed under these conditions.

To examine the intensity of sedation, we used a modification of the scale proposed by Kawamata et al (17). This scale consists of 6 levels as follows: 0 = normal behavior, alert to the environment, standing or grooming; 1 = sitting quietly, sometimes standing or grooming; 2 = sitting quietly, no spontaneous movement, but moved when touched; 3 = no spontaneous movement, moved when touched but flattened themselves within several seconds; 4 = no spontaneous movement, did not move when touched; 5 = loss of righting reflex, unresponsive.

Epidural Dye Spreading and Vehicle Test

Before starting the study, we injected 20 μL Evans blue (Sigma-Aldrich) through the epidural catheter and confirmed that the dye reached the L4-L5 epidural level after sacrificing the rats by transcardial perfusion with 0.1 M phosphate buffer (n = 6). We also injected a 10 μg vehicle through the catheter, and there was no motor dysfunction or behavior change (n = 6).

Statistical Analysis

For analysis of mechanical and thermal responses, the Kruskal-Wallis One way analysis of variance by ranks test, followed by Tukey's test, was used when comparing the control group and RTX-treated groups. For analysis of sedation scores, the same test was used when comparing between groups. The Friedman repeated measures analysis of variance by ranks test, followed by Tukey's test, was used when comparing scores each time after RTX injection within the same group. For obtaining the dose-response curve, we used curve fitting from nonlinear regression by GraphPad Prism 5.04 (GraphPad Software Inc., La Jolla, CA). Data were

expressed as means ± standard deviation. A P value less than 0.001 was considered to be statistically significant. The statistical analysis of the data was all performed by SigmaPlot 11.0 (Sysat Software Inc., San Jose, CA).

RESULTS

The time course of the paw withdrawal threshold to mechanical stimulation is shown in Fig. 1. The withdrawal thresholds of the left hind paws to mechanical stimuli were determined at 15 minute intervals for 180 minutes. RTX ≥ 1 µg groups showed a significant increase in the threshold compared to the control group (P < 0.001). The withdrawal threshold improved to pre-injury level 15 minutes after RTX injection and remained steady for 180 minutes at doses ≥ 1 µg. The withdrawal threshold is dose-dependent up to RTX 2 µg. The further increase in the concentration beyond the 2 µg did not increase the withdrawal threshold.
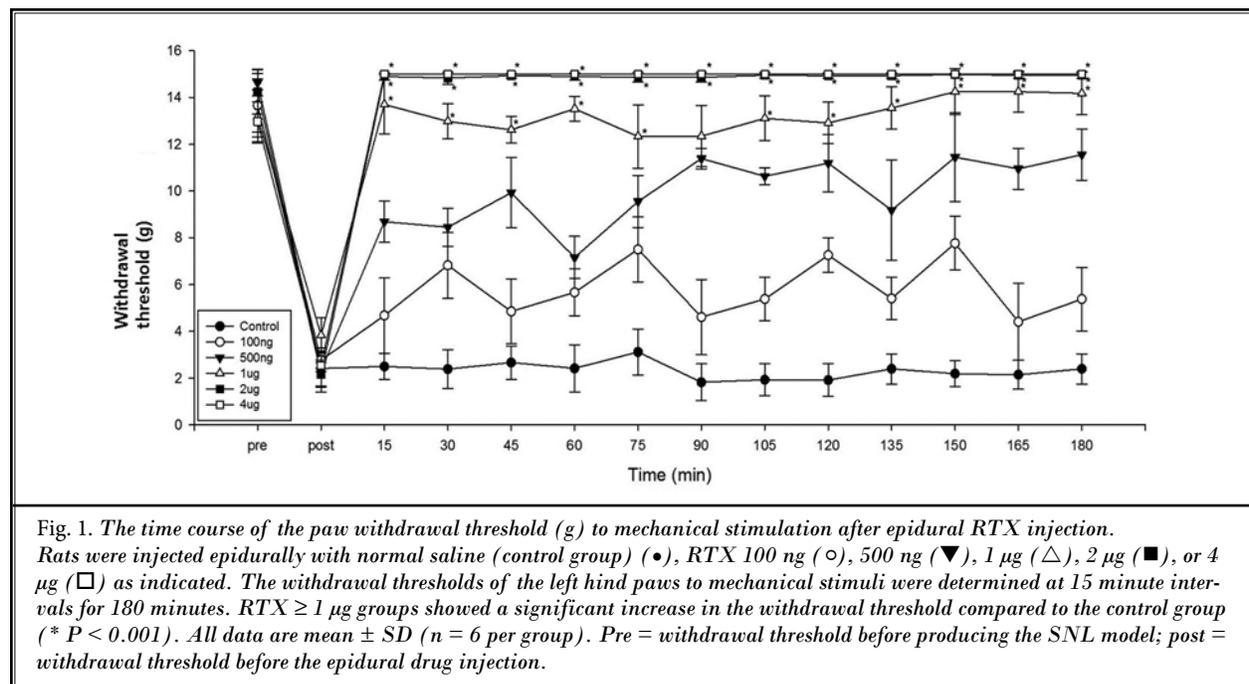
The withdrawal latency to thermal stimuli was determined at 15 minute intervals for 180 minutes (Fig. 2). RTX ≥ 1 µg groups showed a significant increase in withdrawal latency compared to the control group (P < 0.001), but during the first 60 minutes, there were 2 contrasting patterns. The initial paw withdraw latency was lower than the control group for 500 ng and 1 µg groups, while the latency increased significantly for 2

and 4 µg groups. During the interval of decreased latency, rats lifted their paws quickly and licked vigorously after the thermal stimuli. The latency gradually increased for both the 500 ng and 1 µg groups, but the effect of 500 ng abated after 120 minutes.

The sedation score for the initial 180 minutes is shown in Fig. 3. The control group and the 2 lowest doses, 100 ng and 500 ng, had no effect on sedation, while at the doses ≥ 1 µg, the sedation level was dose-dependent (P < 0.001). The RTX 4 µg group showed a statistically significant increase in sedation score compared to the control group during the initial 90 minutes. Afterwards, the sedation level gradually returned to the baseline, independent of the RTX doses.

The behavior changes with respect to RTX concentration are shown in Table 1. All behavior data were based on visual observation. At RTX 1 µg, the rats displayed a very high level of irritability (e.g., shoveling feces, scratching the metal mesh floor nervously with occasional vocalization). The rats exhibited dose-dependent sedation and hyperventilation at doses ≥ 2 µg. At RTX 10 µg, 2 out of 6 rats died, hence doses greater than 10 µg were not evaluated. We did not observe autotomy or motor impairment in any of the RTX-treated rats.
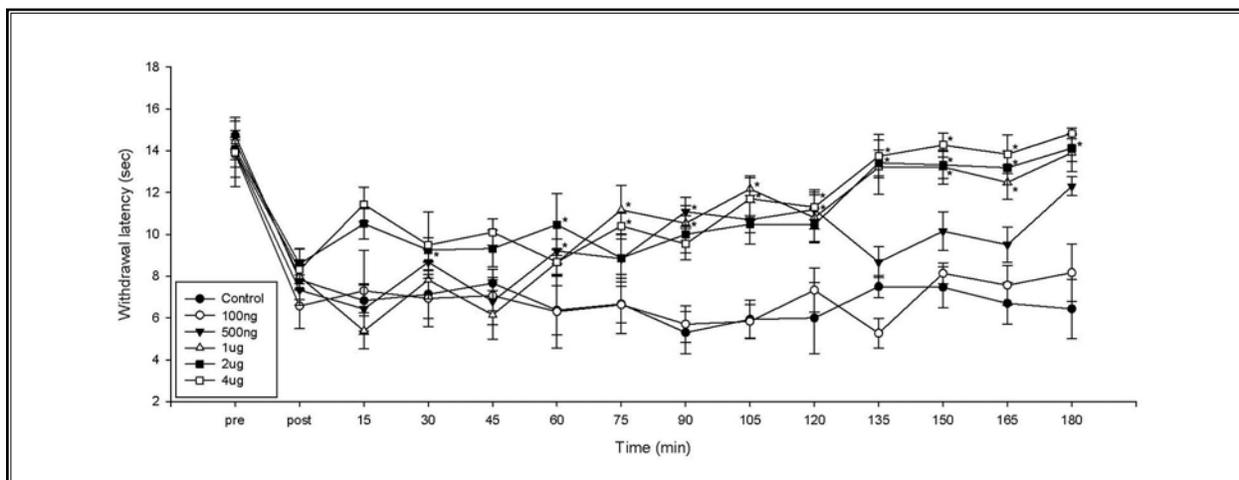
The ED50 for epidural RTX was 265 ng (95% confi-

Fig. 1. *The time course of the paw withdrawal threshold (g) to mechanical stimulation after epidural RTX injection. Rats were injected epidurally with normal saline (control group) (●), RTX 100 ng (○), 500 ng (▼), 1 µg (△), 2 µg (■), or 4 µg (□) as indicated. The withdrawal thresholds of the left hind paws to mechanical stimuli were determined at 15 minute intervals for 180 minutes. RTX ≥ 1 µg groups showed a significant increase in the withdrawal threshold compared to the control group (* P < 0.001). All data are mean ± SD (n = 6 per group). Pre = withdrawal threshold before producing the SNL model; post = withdrawal threshold before the epidural drug injection.*

Fig. 2. *The time course of the paw withdrawal latency (seconds) to thermal stimulation after epidural resiniferatoxin (RTX) administration. Rats were given either normal (control group) (●), RTX 100 ng (○), 500 ng (▼), 1 µg (△), 2 µg (■), or 4 µg (□) as indicated. The withdrawal latencies of the left hind paws to thermal stimuli were determined at 15 minute intervals. RTX ≥ 1 µg groups showed a significant increase in withdrawal latency compared to the control group (* P < 0.001). But during the first 60 minutes, there were 2 contrasting patterns: a hyperalgesic response (RTX 500 ng, RTX 1 µg) and an analgesic response (RTX 2 µg, RTX 4 µg). All data are mean ± SD (n = 6 per group). Pre = withdrawal latency before producing the SNL model; post = withdrawal latency before the epidural drug injection.*
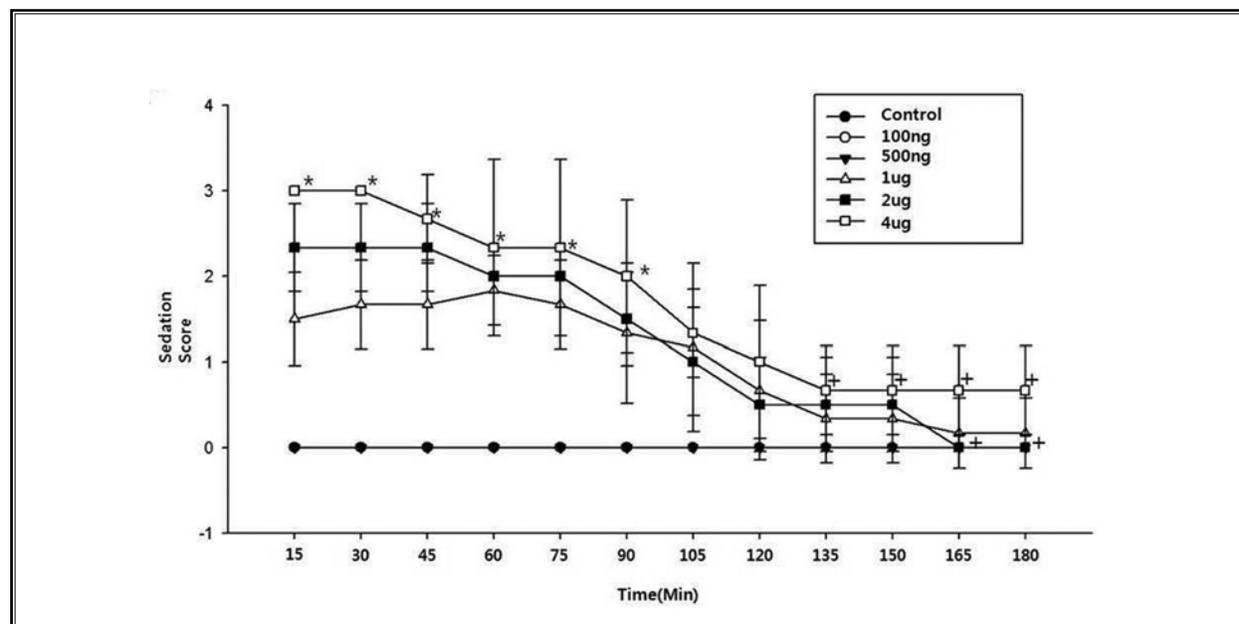

Fig. 3. *The time course of the sedation scores for the first 180 minutes after epidural resiniferatoxin (RTX) administration. The degree of the sedation was assessed at 15 minute intervals using a modification of the scale proposed by Kawamata et al (17). This scale consists of 6 levels, the same as described in the Methods section. The rats were given either normal saline (control group) (●), RTX 100 ng (○), 500 ng (▼), 1 µg (△), 2 µg (■), or 4 µg (□) as indicated. The control, 100 ng, and 500 ng groups did not show any sedation. The RTX 4 µg dose resulted in a significant increase in the sedation score compared to the control group for 90 minutes (* P < 0.001). But the sedation score tended to return to baseline for the groups thereafter; † indicates a significant difference in the sedation score at 15 minutes within the groups. (P < 0.001). All data are mean ± SD (n = 6 per group).*

Table 1. The behavioral changes in the initial 60 minutes after resiniferatoxin (RTX) injection according to RTX dose (n = 6 per group).

RTX Doses	Description of Behavior Changes
100 ng	No change compared to the control group (6/6). *
500 ng	Normal behavior in the eye, no irritable appearance but showed a hyperalgesic response to thermal stimuli (6/6).
1 μg	Frequent voiding and defecation (5/6), shovelling feces, scratching the metal mesh floor nervously (2/6), vocalization (1/6), a hyperalgesic response to the thermal stimuli (6/6).
2 μg	Moving as if in a drunken state with hiccup (2/6), frequent diarrhea (2/6), lying down with hyperventilation (2/6).
4 μg	Moving as if in a drunken state with hiccup (3/6), frequent diarrhea (2/6), lying down with hyperventilation (3/6).
10 μg	Vocalization (5/6), lying down breathing heavily (4/6), vomiting (1/6), death (2/6): one after 8 minutes, the other after 5 hours

* Data represent the number of animals that showed the occurrence out of the total number of animals in the group.
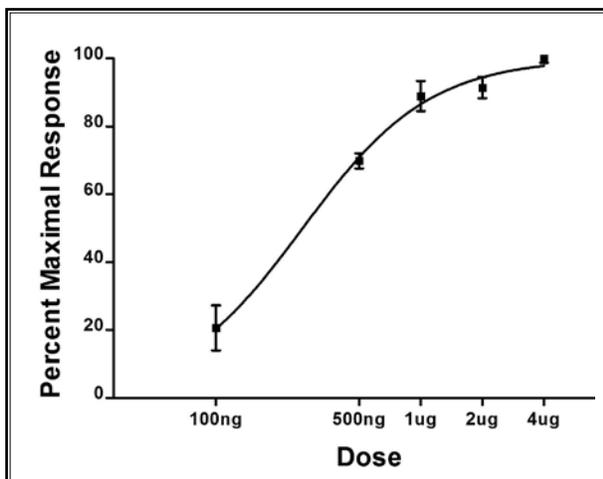

Fig. 4. *The dose response of the hind paw withdrawal latency (seconds) after epidural resiniferatoxin (RTX) administration. The withdrawal latency of the left hind paws was determined by thermal stimulation 180 minutes after RTX injection. The ED50 value for epidural RTX was 265 ng (95% confidence interval, 216.1–324.9 ng) in this experiment. All data are mean ± SD (n = 6 per group).*

dence interval, 216.1–324.9 ng), which was determined from the thermal stimulation response curve shown in Fig. 4. The slope of the curve was consistent with modest cooperativity with a Hill coefficient of 1.436 (95% confidence interval, 1.106–1.694). The ED50 value was estimated at the 180 minutes interval since the analgesic behavior response reached a plateau and showed little variation afterwards.

The long-term mechanical stimulation study (Fig. 5) showed that the paw withdrawal threshold increased significantly during the initial 3-5 days following the RTX administration ($P < 0.001$). However, over the next 15 days, the threshold for the mechanical stimulation approached the baseline, independent of the RTX concentration.

In contrast to the mechanical stimulation, the increased latency to thermal stimulation persisted during the long-term study (Fig. 6). The RTX 2 μg and 4 μg groups showed a statistically significant increase in latency, which was sustained for 20 days ($P < 0.001$). The RTX 1 μg group showed an enhanced analgesic effect for the first 7 days and a considerable, though not statistically significant, analgesic effect for 20 days. The efficacy of RTX 500 ng returned to baseline in 20 days, and RTX 100 ng failed to show any analgesic effect.

The control group showed sustained nociceptive responses of mechanical and thermal stimulation throughout the experiment without restoration, which was consistent with the natural course of neuropathic pain in an SNL rat model (10).

Before we started the experiment, we tested rats in the vehicle group (n = 6) with mechanical and thermal stimuli after RTX injection. There were no differences in nociceptive responses between the normal saline control group and the vehicle group (data not shown).

DISCUSSION

The results of this investigation demonstrate that an increasing withdrawal threshold to mechanical stimulation is not sustained after 3-5 days for any concentrations of epidural RTX, while increasing thermal latency is sustained at doses ≥ 1 μg for 20 days in an SNL rat model. This result has been shown in many experiments using RTX (1,3,4,6,8) and it is consistent with the idea that mechanical sensitivity is carried by large
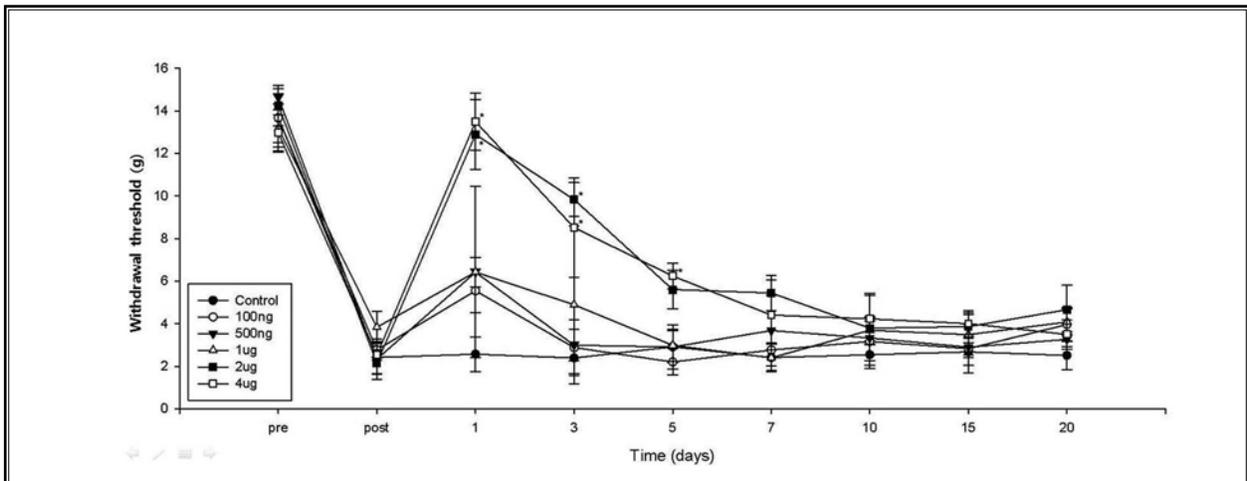
Fig. 5. *The paw withdrawal threshold (g) to mechanical stimulation after epidural resiniferatoxin (RTX) administration for 20 days. Each rat (n = 6 per group) was treated epidurally with either normal saline (control group) (●), RTX 100 ng (○), 500 ng (▼), 1 μg (△), 2 μg (■), or 4 μg (□) as indicated. The withdrawal thresholds of the left hind paws to mechanical stimuli were determined on a daily basis. The 2 μg group and 4 μg groups showed a significant increase in threshold compared to the control group for the initial 3-5 days (* P < 0.001). However, the increased withdrawal threshold returned to the baseline in all RTX doses. All data are mean ± SD (n = 6 per group). Pre = withdrawal threshold before producing the SNL model; post = withdrawal threshold before the epidural drug injection.*
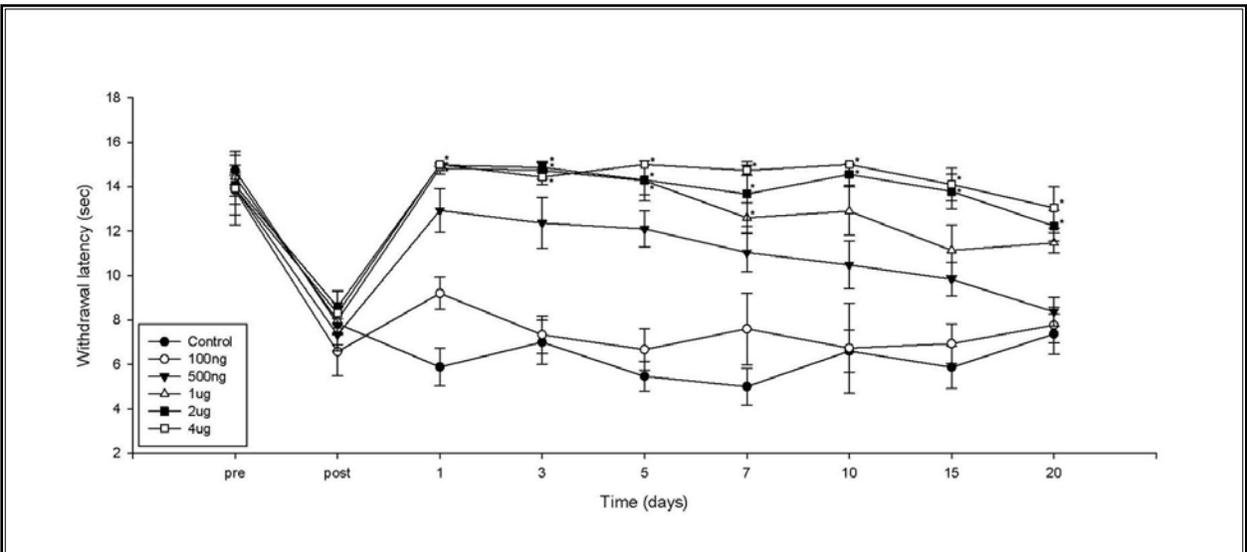

Fig. 6. *The paw withdrawal latency (seconds) to thermal stimulation after epidural resiniferatoxin (RTX) administration for 20 days. Each rat (n = 6 per group) was treated epidurally with either normal saline (control group) (●), RTX 100 ng (○), 500 ng (▼), 1 μg (△), 2 μg (■), or 4 μg (□) as indicated. The withdrawal latency of the left hind paws to thermal stimuli was determined on a daily basis. The RTX 1 μg group showed an increased response compared to the control group for 7 days after receiving RTX. For 2 μg and 4 μg groups, the increase in threshold latency was sustained for 20 days (* P < 0.001). All data are mean ± SD (n = 6 per group). Pre = withdrawal latency before producing the SNL model; post = withdrawal latency before the epidural drug injection.*

Aβ-afferents, not mediated by Aδ- or C- fibers in which TRPV1 is selectively expressed (1).

In the study of perineural RTX on a rat model of postoperative pain, blockade of the heat response lasted for 3 weeks, but blockade of the noxious pressure response did not last longer than 48 hours, even with the maximum (10 μg) concentration (6). On the other hand, other RTX studies on a rat model of inflammatory pain have described no differences in response to the von Frey test when comparing control to RTX-treated groups. It has been considered as a merit, because it has been interpreted as maintaining normal proprioceptive sensation with selectively blocking inflammatory-induced thermal hyperalgesia (1,4,8).

This phenomenon has also been shown on a rat model of neuropathic pain (9,18,19), but it has been considered as a disadvantage because neuropathic pain has a characteristic of mechanical allodynia, which means feeling pain from stimuli which are not normally painful (9,19).

Enhanced behaviors to tactile stimulation after nerve injury can be modulated either through a spinal pathway or by interfering with the dorsal column (e.g., touch) pathway (19). Therefore, whether an exaggerated response to the von-Frey test in SNL-rats is an indication of pain (e.g., allodynia) has been questioned. Tactile hypersensitivity is still present in SNL-rats even after functional desensitization of TRPV1-positive neurons with RTX, so there has been debate about whether the interpretation of the phenomenon is the failure in blocking mechanical allodynia or just a nonpainful hypersensitive state (19). Consequently, we used the term "mechanical hypersensitivity" instead of allodynia. Whatever the mechanism, we found transitory improvement of mechanical hypersensitivity in SNL-rats with epidural RTX administration.

The most important advantage of epidural RTX injection in a neuropathic pain model is the dose-sparing effect. There have been the studies of systemic RTX injection that resulted in a significant and long-lasting decrease in sensitivity to thermal stimuli in an SNL rat model (9,19). But they used a relatively large amount (300 μg/kg subcutaneously or 100 μg/kg, intraperitoneally) of RTX compared to us. Meanwhile, there is a report that the thermal analgesic effect of RTX on an SNL model lasted for a relatively short period (not much longer than 24 hours), in which perineural RTX of 500 ng was used (18). We think that the dose and the route of administration are responsible for these different results.

Kissin et al (18) also described that the therapeutic effect of RTX on already developed neuropathy was inferior to a preventive effect prior to nerve injury. But our data suggested that even treatment of RTX on already developed neuropathy had a dose-dependent decrease of tactile hypersensitivity even though it was transitory, and sustained, profound thermal analgesic effect when using the epidural route. A difference between the study above and our experiment is that mechanical hyperalgesia was measured by von Frey filament of 12.0 g (18), whereas we used von Frey filaments of 0.4 - 4.0 g for evaluating mechanical hypersensitivity. Postinjury treatment becomes necessary anyway since preemptive therapy cannot be possible in all clinical situations.

A previous report studied epidural RTX in normal rats, which produced profound thermal analgesic effect over 7 days (7). The authors used a relatively large amount of RTX (300 μg/kg), and the ED50 of thermal hyperalgesic response was 5.9 ± 0.3 μg/kg, which was much higher than our results (7). This represents that the neuropathic pain conditions have a low sensitivity threshold to thermal stimulation and a shift to the left on the dose-response curve compared with normal conditions.

In clinical settings, searching for the minimum effective analgesic dose is important for reducing potential side effects (8). Our results showed that the epidural dose needed to adequately blunt the thermal stimulation is at least a 1 μg dose, which was lower than systemic doses of other reports (7,9).

A drawback to the use of epidural RTX 1 μg is aggressive behaviors with the initial hyperalgesic response in RTX-treated rats. It was transient and faded with time, and did not reappear at higher doses. We thus concluded that this phenomenon was a result of a reaction against pain, not systemic effect (5, 20). There have been several animal studies describing similar behaviors (3,5,7). There were no reports of abnormal behavior changes even with intracisternal RTX administration in dogs (4).

Another problem is dose-related sedation. Even the RTX-treated rats in peripheral nerve endings have shown sedation at doses of strong localized analgesic effects (0.0625-0.25 μg) (3), which was similar in our experiment (1.0-4.0 μg). Kissin et al (20) described short-lasting (up to 90 minutes) sedation in RTX-treated rats as an acute systemic toxicity. They explained that such changes were not obvious with preliminary bupivacaine injection. The epidural route of administration provides an enhanced localized effect, but cannot completely

prevent the gradual systemic absorption of RTX (7). We observed hyperventilation and agonized respiration in the RTX ≥ 2 μg groups, which was not observed in all rats of the RTX 1 μg group. So the effect of RTX ≥ 2 μg is not more advantageous than that of RTX 1 μg.

Our limitations are a relatively short duration of observation (20 days) and no histological examination. The 20-day observation period was based on the results of recovery of sciatic nerve-damaged rats, in which regeneration of the sciatic nerve began to contribute to nociception no earlier than 3 weeks after nerve damage (6). So, the recovery of nociceptive responses before this time means a dropped analgesic effect, not nerve regeneration (6). Applying the above-mentioned criteria, RTX ≥ 2 μg in our study seems to have an irreversible effect. We did not perform a histological study, but there has been a report of no significant histological damage to unmyelinated nerve fibers with RTX 1 μg by perineural application (21).

TRPV1 receptors are known to be highly expressed on nociceptive neurons of the dorsal root ganglia but are also known to have a moderate expression on the brain and spinal cord (22). Although TRPV1 is present throughout the whole brain area, little is known about its function in the central nervous system. Therefore, the TRPV1 receptor may play a much wider role than pain perception and can increase the possibility for un-anticipated side effects (23,24).

RTX produces an initial depolarization of primary sensory neurons, including TRPV1 receptors, which causes pungency and irritation followed by a long-lasting desensitization of the nerve; this may be due to calcium accumulation in the neurons, which causes calcium cytotoxicity (4,5,9). Therefore, the initial excitation phase is obligate for analgesic response, so RTX should be administered with an anesthetic (5). General anesthesia was required for intrathecal RTX administration in dogs with bone cancer, and bupivacaine injection prior to RTX has been shown to reduce pain in rats (3,5,6). We did not use any local anesthetics prior to RTX injection since the application could confound the results (9). Consequently, the initial excitation response of RTX has made it problematic to implement the wide use of RTX, which should be resolved (3,25).

In conclusion, RTX has a potential to be used in a rat model of neuropathic pain with a relatively low dose via epidural administration, which produces transitory improvement of mechanical hypersensitivity and prolonged thermal analgesic response. But further in-depth studies to evaluate the margin of safety is warranted before initiating a clinical trial.

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