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COMPARISON OF THE RECOVERY PROFILE OF HYPERALGESIA EVALUATED BY THREE DIFFERENT METHODS AND EFFECTS OF INTRATHECAL MORPHINE IN POSTOPERATIVE PAIN MODEL OF RATS

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ABSTRACT

Background: We compared the effects of paw incision, intrathecal single or repeat morphine administration on three different tests of hyperalgesia using a rat model to know the characteristics of these tests. **Methods:** Male Sprague-Dawley rats with lumbar intrathecal catheters received a 1 cm longitudinal incision through skin and fascia of the plantar aspect of the foot followed by suture. Von Frey filament withdrawal weighting, thermal withdrawal latency, and weight bearing were evaluated for postoperative 7 days. The effects of single intrathecal morphine after surgery and repeat intrathecal morphine every day on the three methods were also studied. **Results:** Paw incision decreased Von Frey withdrawal weighting, thermal withdrawal latency, and weight bearing only on the operated paw. In these methods, Von Frey withdrawal weighting and thermal withdrawal latency gradually returned after 2 hours, but not to the control levels on 7 days. Single intrathecal morphine recovered Von Frey withdrawal weighting and thermal withdrawal latency dose dependently, but not weight bearing. Repeated intrathecal morphine every day induced similar recovery with single intrathecal morphine in the Von Frey withdrawal weighting, and better recovery in the thermal withdrawal latency. **Conclusions:** To study postoperative analgesia in a rat paw incision model, weight bearing showed different results compared to Von Frey withdrawal weighting and thermal withdrawal latency. Repeated intrathecal morphine every day after surgery induced better recovery of thermal hyperalgesia but not of tactile mechanical hyperalgesia than postoperative single intrathecal morphine.

KEYWORDS: Postoperative pain, rat, hyperalgesia, intrathecal, morphine.

INTRODUCTION

A surgical incision model of rats by Brennan et al.[1] is often used to study postoperative pain. Von Frey filament withdrawal response or thermal withdrawal response is used to analyze hyperalgesia induced by incision. Weight bearing is one of the tests to investigate mechanical hyperalgesia. Postoperative hyperalgesia evaluated by three different methods might have different profiles. However, there are no studies to compare these three methods after plantar incision of rats. The first purpose of this study was to know the different recovery profiles of hyperalgesia evaluated by three different methods in a rat incision model.

Intrathecal morphine had analgesic effects on postoperative pain in a rat model.[2] However, morphine prolonged postoperative pain which is associated with increased expression of inflammatory genes.[3] Therefore, the second purpose was to know the effects of intrathecal morphine on postoperative hyperalgesia evaluated by three different methods.

Repeated administration of morphine can exacerbate pain in inflammatory and neuropathic pain models.[4,5,6] Then, the third purpose was to know whether repeated administration of intrathecal morphine decreased analgesic effects or not in a rat incision model.

MATERIALS AND METHODS

Intrathecal catheterization

After obtaining the approval of the Research Committee of the University of Tokyo, male Sprague-Dawley rats (280-300 g; Nippon Bio-Supply, Tokyo, Japan) were implanted with lumbar intrathecal catheters under halothane (2 %) anesthesia. The procedures are the same as our previous study.[7] Briefly, an 8.5 cm polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ) was inserted caudally to the thoracolumbar level in the

intrathecal space through atlanto-occipital membrane. The rostral part of the catheter was plugged with a 28-gauge steel wire and put through to the top of the skull. Only rats with normal motor function and behavior and increase in body weight seven days later were used for experiments.

Surgical procedure

Rats with lumbar intrathecal catheters were anesthetized with halothane. A 1 cm longitudinal incision was made through skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel as the method by Brennan et al.[1] The plantaris muscle was elevated. The skin was sutured with two mattress sutures of 5-0 nylon.

Evaluation of hyperalgesia

Before surgery and 2, 24, 48, 72, 96, 120, 144, and 168 hours after surgery, withdrawal response to von Frey filament application, thermal withdrawal response to light beam, and weight bearing were evaluated on both paws.

To evaluate tactile mechanical hyperalgesia, von Frey filaments (0.008 – 300 g) were applied from underneath the cage through openings in the wire mesh floor to the area adjacent to the wound on the operated paw and to the same area on the non-operated paw. Each filament was applied starting with 0.04 g and continuing until a withdrawal response occurred. The test was performed three times in each time point. A withdrawal response was considered to be complete lifting of the hind paw off the surface of the cage or flinching. The lowest force in three measurements was considered to be the withdrawal threshold.

To evaluate thermal hyperalgesia, thermal withdrawal response to radiant heat was evaluated. A rat was placed in a clear plastic chamber on a glass floor where rat can move with free. A radiant heat source (halogen projector lamp CXL/CXP 50 W 8V, Ushio Tokyo, Japan) was focused on the plantar surface of one hind paw and applied to the area adjacent to the wound on the operated paw and to the same area on the non-operated paw. The time interval between the application of light beam and hind paw withdrawal response was measured.

To evaluate spontaneous hyperalgesia, weight bearing test was performed. A rat is located in a holder specially designed to maintain the rat comfortably on two separate sensor plates. The Incapacitance device (Ugo Basile, Milan, Italy) enables to quantify the spontaneous postural changes reflecting spontaneous pain by independently measuring the weight that the animal applies each hind paw. Because normal rats distribute weight equally on both paws, change of this equilibrium can reflect the level of discomfort due to an injured paw.

Protocol 1. Effects of incision

Each eight rats with paw incision were evaluated with three methods.

Protocol 2. Effects of postoperative intrathecal morphine administration

After suture, saline (control), morphine 1, 3, or 10 µg in 10 µl saline was administered intrathecally and three methods were evaluated in each eight rats.

Protocol 3. Effects of repeat administration of intrathecal morphine

After suture, and 10 minutes before each test every day, saline (control), morphine 1, 3, or 10 µg in 10 µl saline was administered intrathecally, then three methods were evaluated in each eight rats.

Statistics

For statistical analysis, repeated analysis of variance was used and $P < 0.05$ was considered to be statistically significant.

After the study, rats were euthanized under halothane 5% and the location of the catheter was confirmed anatomically and the data of the rats with mal location of the catheter was excluded, and another rat was added to fill the number of each group.

RESULTS

Protocol 1

Non-operated paw did not show any changes in all three tests during the study period (Fig.1,2,3). Von Frey withdrawal weighting (Fig.1), thermal withdrawal latency (Fig.2), and weight bearing (Fig.3) significantly decreased in the operated paw. The former two gradually recovered but were still significantly lower than the control value and the values of the non-operated paw in 7 days (Fig. 1,2). The measured values in the operated paw of all three tests were significantly lower than those in the non-operated paw in 7 days (Fig. 1,2,3).

Protocol 2

Intrathecal morphine increased von Frey filament withdrawal weighting dose dependently in the operated paw (Fig.4), but no differences by morphine were found in the non-operated paw (Fig.5). Thermal withdrawal latency decreased in the operated paw but increased with morphine dose dependently (Fig.6). In the non-operated paw, thermal withdrawal latency did not change with or without morphine (Fig.7)

Weight bearing did not change in both the operated paw (Fig.8) and the non-operated paw by morphine (Fig.9).

Protocol 3

Von Frey filament withdrawal weightings in the operated paw were higher dose dependently with repeat morphine until 5 days (Fig.10). In the non-operated paw, control

values of withdrawal weightings were different among the doses, therefore, we could not compare thereafter (Fig.11). However, no significant changes were observed in any doses. Thermal withdrawal latency of the operated paw decreased for 7 days with saline, but only at 2 hours with morphine 1, and 3 µg (Fig.12). Morphine induced higher thermal withdrawal latency than saline in the operated paw (Fig.12). Thermal paw withdrawal latency did not change significantly in the non-operated paw (Fig.13). In the operated paw, control values of the weight bearing showed significant differences between morphine 1 µg and 3µg with saline (Fig.14). Therefore, we could not compare thereafter among the doses. In the non-operated paw, weight bearing did not show significant differences among the groups (Fig.15)

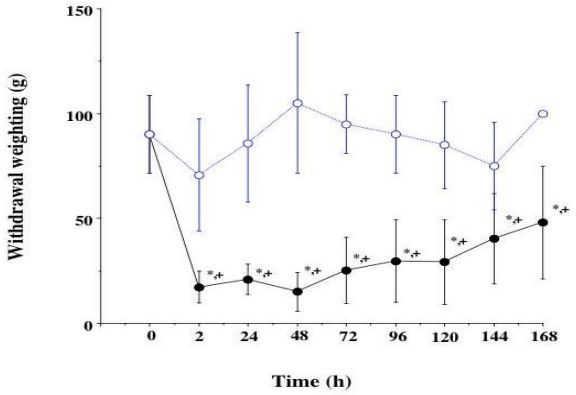

Figure 1: Effects of surgery on Von Frey filament withdrawal response Closed circle, operated paw; open circle, non-operated paw; bars, standard deviation *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. non-operated paw.

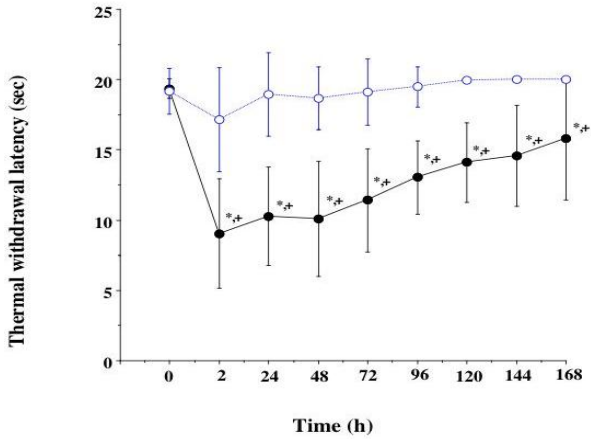

Figure 2: Effects of surgery on thermal paw withdrawal latency Closed circle, operated paw; open circle, non-operated paw; bars, standard deviation *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. non-operated paw.

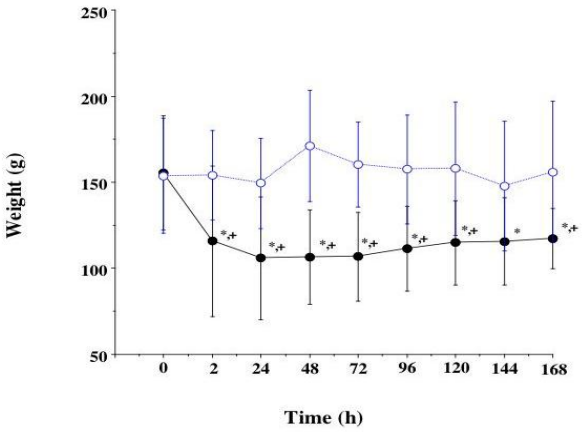

Figure 3: Effects of surgery on weight bearing Closed circle, operated paw; open circle, non-operated paw; bars, standard deviation *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. non-operated paw.

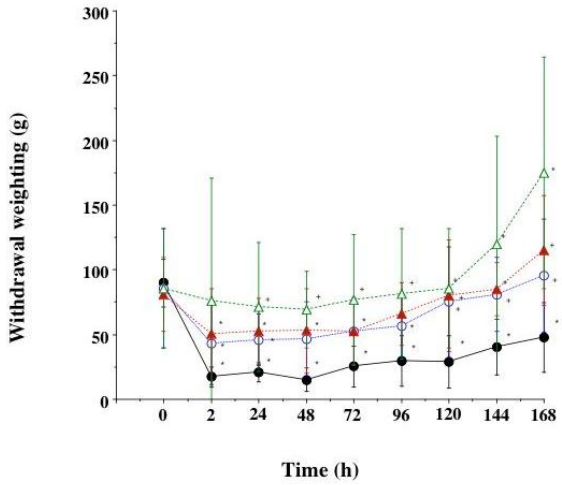

Figure 4: Effects of intrathecal single morphine on Von Frey filament withdrawal response; operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation; *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. saline.

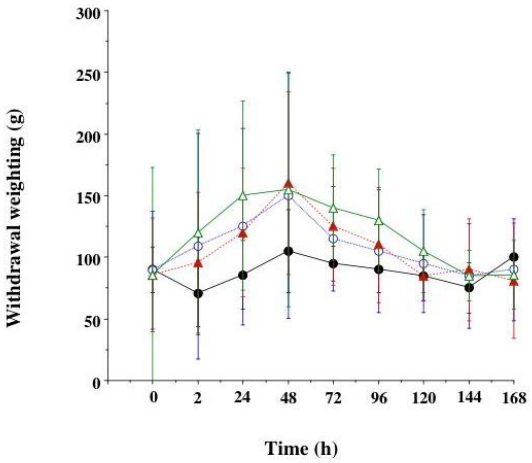

Figure 5: Effects of intrathecal single morphine on Von Frey filament withdrawal response; non-operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation.

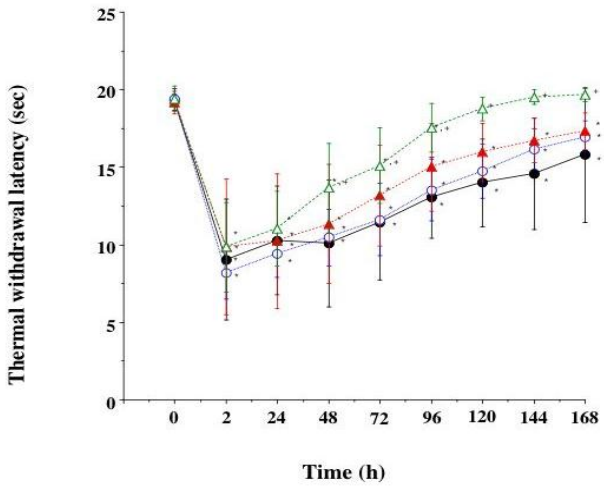

Figure 6: Effects of intrathecal single morphine on thermal paw withdrawal latency; operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation; *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. saline.

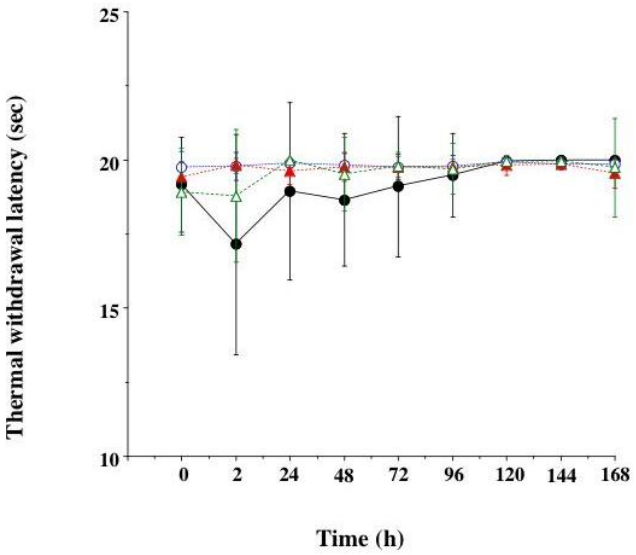

Figure 7: Effects of intrathecal single morphine on thermal paw withdrawal latency; non-operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation.

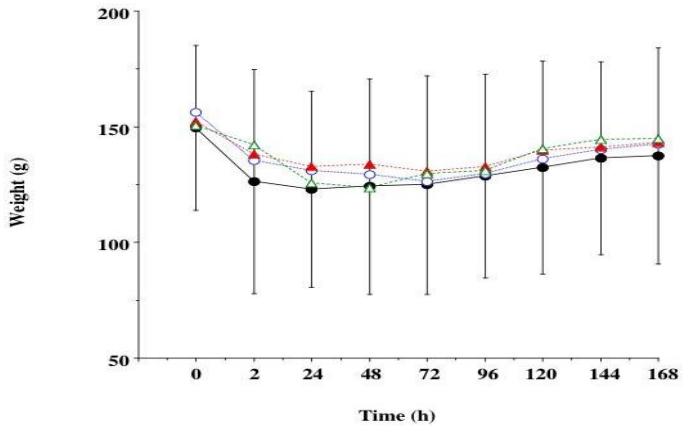

Figure 8: Effects of intrathecal single morphine on weight bearing; operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation

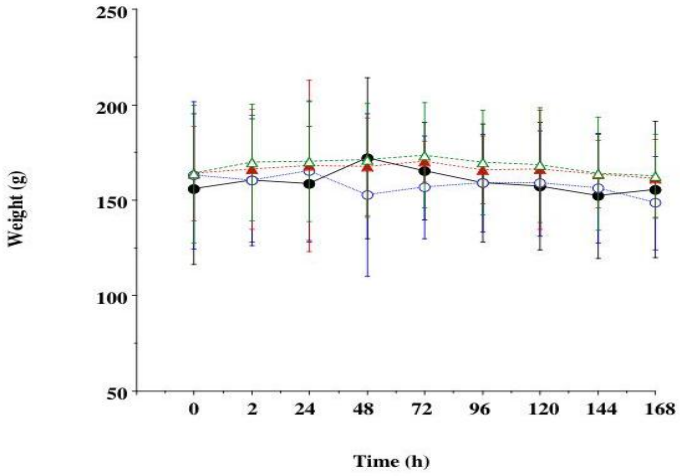

Figure 9: Effects of intrathecal single morphine on weight bearing; non-operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation.

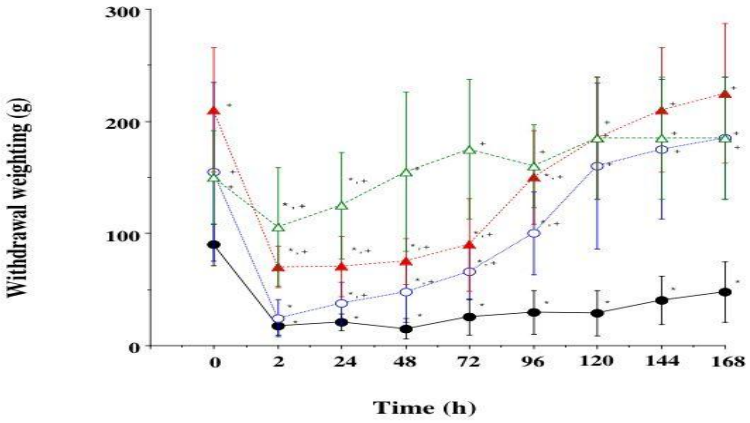

Figure 10: Effects of intrathecal repeat morphine on Von Frey filament withdrawal response; operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation; *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. saline.

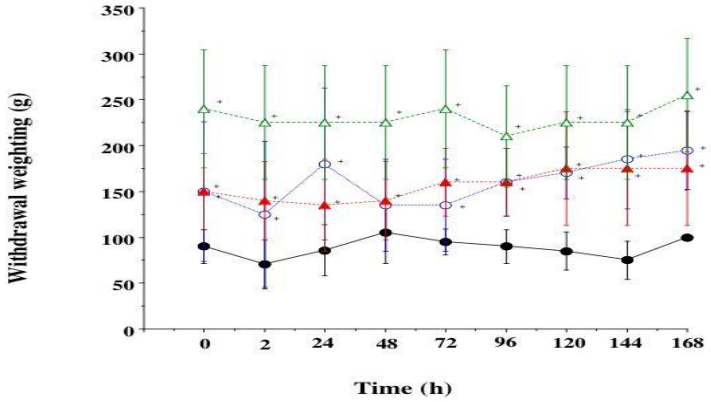

Figure 11: Effects of intrathecal repeat morphine on Von Frey filament withdrawal response; non-operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation; +: P < 0.05 vs. saline.

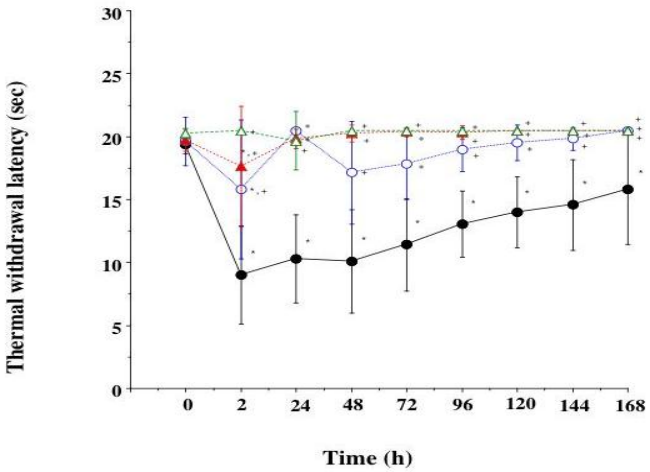

Figure 12: Effects of intrathecal repeat morphine on thermal paw withdrawal latency; operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation; *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. saline.

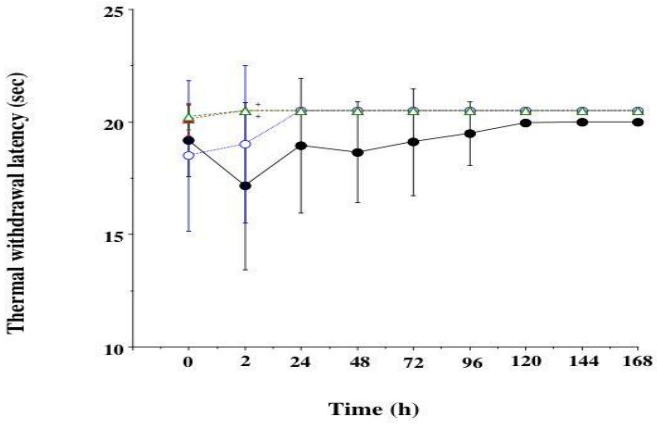

Figure 13: Effects of intrathecal repeat morphine on thermal paw withdrawal latency; non-operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation; +: P < 0.05 vs. saline.

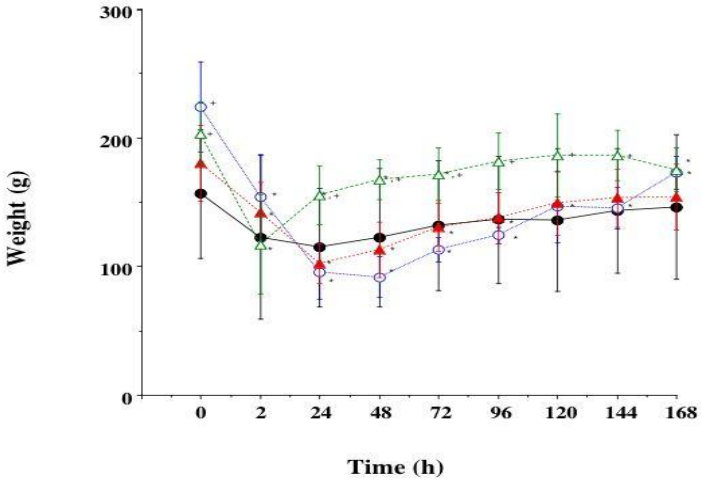

Figure 14: Effects of intrathecal repeat morphine on weight bearing; operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation; *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. saline.

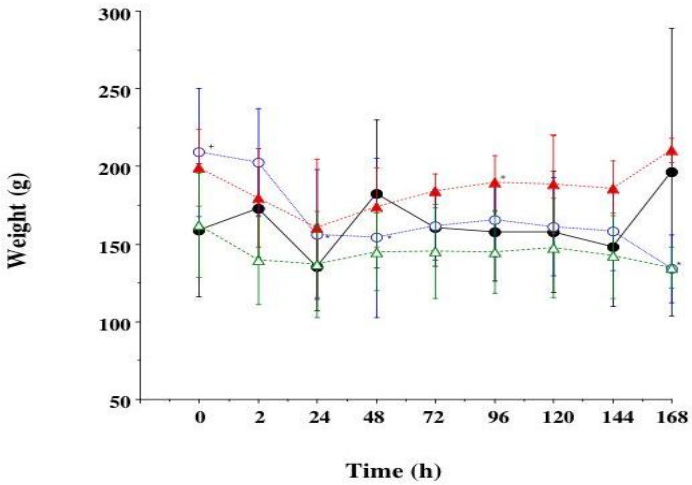

Figure 15: Effects of intrathecal repeat morphine on weight bearing; non-operated paw Closed circle, saline; open circle, 1µg; closed triangle, 3µg; open triangle, 10µg; bars, standard deviation; *: P < 0.05 vs. the value at time 0; +: P < 0.05 vs. saline.

DISCUSSION

Paw incision decreased von Frey withdrawal weighting, thermal withdrawal latency, and weight bearing only on the operated paw. In these methods, von Frey withdrawal weighting and thermal withdrawal latency gradually returned after 2 hours, but not to the control levels in 7 days. Single intrathecal morphine recovered von Frey withdrawal weighting and thermal withdrawal latency dose dependently, but not weight bearing. Repeated intrathecal morphine every day induced similar recovery with single intrathecal morphine in the von Frey withdrawal weighting, and better recovery in the thermal withdrawal latency.

Surgery induces two phases of sensory input in the acute post-operative period, first in response to tissue damage from the incision and second, an inflammatory reaction to the tissue damage involving release of pro-inflammatory mediators at the site, induction of peripheral and central sensitization, and hypersensitivity.[8] The use of mechanical allodynia is an elicited response and a measure of pain. However, it does not give a true reflection of painful input into the central nervous system from the injured site. It provides objective information regarding the sensitivity of the nerves in the injured area, although it may be slightly different from the pain signals at rest. The use of thermal hyperalgesia is a measure of C-fiber mediated pain.[9] However, the correlation between postoperative pain sensation and thermal hyperalgesia is unclear. Primary and secondary hyperalgesia after an incision were not modulated by descending influence from the rostral medial medulla.[10] Thus, incision induced pain involves dissimilar mechanisms compared with inflammatory and neuropathic pain. However, no other tests exist to measure incision induced pain in animal models.

It is possible that the patterns and modes of the transmission of nociceptive information from thermal and mechanical nociception to the spinal cord are different. Mechanical hyperalgesia, tactile allodynia, and a decrease in weight bearing were present on the affected limb within 1 day of plantar surface incision with maximum sensitivity 1 – 3 days postoperatively and resolved in 3 days by Whiteside et al.[11] Brennan et al. showed that the paw incision model displays up to 4 days of mechanical hypersensitivity.[1] Reduced heat withdrawal latency is greatest the day of incision and sustained for about 5 days and is completely resolved by 7 to 10 days after surgery. Withdrawal threshold to von Frey application for mechanical testing is markedly decreased and this response is sustained for 5 to 10 days in another study by Brennan et al.[12] The duration of prolonged spontaneous foot lifting was highest at 3 hours after incision and steadily diminished in 3 days.[13] Our study investigated for only 7 days, but von Frey withdrawal response, thermal withdrawal latency, and weight bearing did not recover in 7 days, which were similar with Brennan et al.,[12] but not with Kabadi et al.[13]

Our results showed postoperative single intrathecal morphine was effective on von Frey withdrawal weighting and thermal withdrawal latency not on weight bearing, but only 10 μg had significant effects until 4 days after surgery.

When intrathecal morphine was administered every day, 1 to 10 μg had significant effects on von Frey withdrawal weighting and thermal withdrawal latency until the end of the study period. However, we could not evaluate its effects on weight bearing because control values showed significant differences among the doses.

A hind paw plantar incision induces spontaneous pain like behaviors, which are more sensitive to morphine than mechanically evoked behaviors.[13] The lower doses of morphine attenuated prolonged spontaneous foot lifting but not mechanical hyperalgesia.[13] Our results did not confirm these because single administration did not have any changes in the weight bearing and repeat administration seemed to have greater effects on von Frey withdrawal weighting than weight bearing.

Intrathecal morphine has about 120 minutes duration of analgesic action on acute surgical pain in rat.[14] Thomas et al. showed that intrathecal morphine 0.2 mg/kg had analgesic effects up to 8 hours in a rat model of caudal laparotomy.[2] Pre or post incisional morphine administration prevented early pain behaviors but had no long-lasting effects on subsequent hyperalgesia in rat incisional model by Brennan et al.[15] Our results had 4 days duration of analgesia in mechanical and thermal hyperalgesia with single intrathecal morphine. These studies had quite different duration of analgesia, which may be due to different surgical procedure and different morphine doses.

Preoperative morphine prolongs postoperative pain by increasing proinflammatory cytokines.[3] Repeated morphine enhances pro-inflammatory response when measured within 2 hours of the last dose of morphine.[16] Thus, repeated morphine might decrease analgesic effects. In normal rats, acute tolerance after systemic morphine occurred in 5 to 7 days.[17] Neuraxial morphine requires longer exposure to produce tolerance. Therefore, 7 days in our study might be shorter to investigate tolerance of repeat morphine.

CONCLUSIONS

To study postoperative analgesia in a rat paw incision model, weight bearing showed different results compared to von Frey withdrawal weighting and thermal withdrawal latency. Repeated intrathecal morphine every day induced better recovery of thermal hyperalgesia but

not of tactile mechanical hyperalgesia than postoperative single intrathecal morphine.

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