Original Article

DOI: 10.5582/bst.2018.01037

Effects of three forms of local anesthesia on perioperative fentanyl-induced hyperalgesia

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Summary **Both local infiltration analgesia (LIA) and nerve block are common analgesic modalities for pain relief after surgery. The aim of the current study was to investigate the effects of those two modalities on pain behavior and the expression of pro-inflammatory cytokines such as interleukin (IL)-1β and IL-6 and tumor necrosis factor-α (TNF-α) in the spinal cord and dorsal root ganglion (DRG) in a rat model of perioperative fentanyl induced hyperalgesia. Rats were injected with fentanyl (60 μg/kg) 4 times and received a plantar incision after the second injection or they received pre-incision LIA and sciatic nerve block (SNB) or post-incision LIA with levobupivacaine (0.5%, 0.2 mL). Mechanical and thermal nociceptive thresholds were assessed using the tail pressure test and paw withdrawal test on the day before drug injection, 1 and 4 hours after injection, and 1-7 days later. The lumbar spinal cord and dorsal root ganglia were collected from rats in each group to measure IL-1β, IL-6, and TNF-α on the day before drug injection, 4 hours after injection, and 1, 3, 5, and 7 days later. Fentanyl and an incision induced a significantly delayed mechanical hyperalgesia in the tail and thermal hyperalgesia in both hind paws and up-regulation of pro-inflammatory cytokines in the spinal cord and dorsal root ganglia. Rats treated with pre-incision LIA and SNB or post-incision LIA had alleviated hyperalgesia and significantly reduced levels of IL-1β, IL-6, and TNF-α compared to the control group. LIA and SNB partly prevented perioperative fentanyl-induced hyperalgesia and up-regulation of pro-inflammatory cytokines in the spinal cord and dorsal root ganglia.**

Keywords: Regional anesthesia, hyperalgesia, spinal cord, dorsal root ganglion, pro-inflammatory cytokines

1. Introduction

Postsurgical pain is a common complication of many surgical procedures and the most frequent cause of discomfort after surgery (*1*). Cutting and handling tissue during surgery causes trauma and inflammation that in turn activate nociceptors and induce postsurgical pain (*2*). Opioids, used perioperatively as a basal analgesic, cause opioid-induced hyperalgesia (OIH) that may aggravate postsurgical pain (*3*).

Peripheral nerve block, such as local infiltration anesthesia (LIA) and regional nerve block, can enhance perioperative pain control and may have more positive effects (*4,5*). To the extent known, however, previous studies have rarely simultaneously investigated the effect of local anesthesia on a surgery-induced increase in pro-inflammatory cytokines in the spinal cord and dorsal root ganglia. Since the activation of spinal glia and upregulation of pro-inflammatory cytokines are critical to the development of post-surgical pain (*6,7*), the aim of the current study was to investigate the effects of LIA and sciatic nerve block (SNB) on pain behavior and

Released online in J-STAGE as advance publication April 15, 2018.

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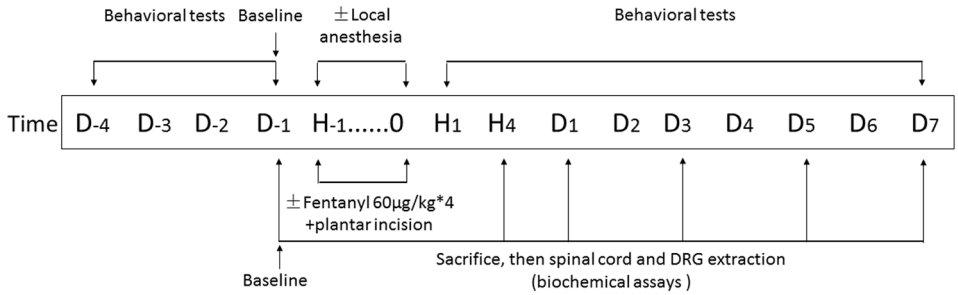

Figure 1. Medication and Measurement Protocol. Rats were randomly assigned into 5 groups (n = 28 in each group). Rats in the normal group received no treatment. The other 4 groups of rats were subcutaneously (s.c.) injected with fentanyl at a dose of 60 μg/kg 4 times at 15-minute intervals and received a plantar incision after the second injection. Rats in 3 groups (treatment groups) received pre-incision local infiltration anesthesia (LIA) or sciatic nerve block (SNB) or post-incision LIA with 0.2 ml of 0.5% levobupivacaine respectively. Behavioral tests were performed daily for 11 days (D-4 to D-1 and D1 to D7) and 1 and 4 hours (H1 and H4) after treatment. The thermal and mechanical thresholds on D-1 were recorded to serve as baseline values. The lumbar spinal cord (L3-L6) and both ipsilateral and contralateral dorsal root ganglia (L3-L6) were collected on D-1, H4, D1, D3, D5, and D7 to examine the expression of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor (TNF-α).

the expression of pro-inflammatory cytokines such as interleukin (IL)-1β and IL-6 and tumor necrosis factor-α (TNF-α) in the spinal cord and and dorsal root ganglia of rats. Given the fact that opioids are indispensable analgesics in most surgeries, this study used a rat model of perioperative fentanyl-induced hyperalgesia.

2. Materials and Methods

2.1. *Animals*

This study was approved by the Institutional Laboratory Animal Care and Use Committee of Sun Yat-sen University and was carried out in accordance with the guidelines of the Chinese Physiological Society regarding the care of experimental animals. Adult male Sprague Dawley rats weighing 200-250 g (purchased from Sun Yat-sen University, Guangzhou China) at the start of the experiments were used. All rats were housed 4 animals per cage in a specific pathogen-free environment at 25 ± 2 ℃ with a 12-hour light/dark cycle. Food and water were available ad libitum. Rats were randomly assigned into 5 groups (n = 28 per group), including a normal group (N), a fentanyl and a plantar incision (FI) group, a pre-incision LIA (Pre-L) group, a pre-incision SNB (Pre-S) group, and a post-incision LIA (Post-L) group.

2.2. *Medication and measurement protocol*

Rats in the normal group received no intervention. The four other groups of rats received subcutaneous injections of fentanyl (60 μg/kg) 4 times at 15-minute intervals and underwent surgery after the second injection. Rats in the Pre-L group and the Pre-S group received LIA or an SNB with a single dose of levobupivacaine (0.2 mL, 0.5%) before a plantar incision and rats in the Post-L group received LIA after a plantar incision. Behavioral tests as described below were performed daily for 11 days (D-4 to D-1, D1 to D7) and 1 and 4 hours (H1 and H4) after fentanyl injections on the day of treatment. Behavioral tests were performed on D-4 to D-2 to acclimate rats to the apparatus and environment. Values on D-1 before treatment were recorded as baseline values. All behavioral tests were performed by a single investigator blinded to the treatment groups. Each test (between 9:00 and 14:00) was repeated 3 times at 3-minute intervals, and results were averaged to obtain a final value. The lumbar spinal cord (L3-L6) and dorsal root ganglia (L3-L6) were collected on D-1, H4, D1, D3, D5, and D7 to examine the expression of IL-1β, IL-6, and TNF-α (Figure 1).

2.3. *Surgery and local SNB*

2.3.1. *Surgery*

Rats in the FI, Pre-L, Post-L, and Pre-S groups were anesthetized with 1.5% to 2% isoflurane (Hebei Jiupai Pharmaceutical Co., Hebei, China). A 1-cm incision was made through the skin, fascia, and muscle of the plantar aspect of the left hind paw after skin disinfection. The incision was started 0.5 cm from the proximal edge of the heel and extended to the toes (*8*). A 4-0 suture was used to suture the skin after the wound was dabbed with a cotton swab to stop bleeding. All incisions were made after the second injection of fentanyl.

2.3.2. *LIA*

Rats in the Pre-LIA group and the Post-LIA group respectively received LIA on the left before or after a plantar incision following anesthesia with 1.5% to 2% isoflurane. The needle was inserted about 4 mm, and levobupivacaine (0.2 mL, 0.5%) was administered slowly over 5 seconds when no bleeding occurred.

2.3.3. *SNB*

Rats in the Pre-S group received an SNB on the left

before a plantar incision after anesthesia with 1.5% to 2% isoflurane. The greater trochanter was located by palpation in the left hind limb, and a needle (26G) was introduced slowly from the left femoral shaft into the sciatic notch. If slight contraction, shaking, or spasming of the left leg was noted, the needle was deemed to be close to the sciatic nerve (*5*). To avoid nerve damage, the needle was pulled out about 1 mm, and levobupivacaine (0.2 mL,0.5%) was administered slowly over 5 seconds when no bleeding occurred. Rats with the toes and foot plantar flexed with no splaying 5-10 minutes after the SNB and without dyskinesia in the left hind limb 1 day after SNB were used in experiments.

2.4. *Behavior tests*

2.4.1. *Tail pressure test*

The mechanical threshold of a response in the tail was evaluated with an electronic device (ZH-YLS-3E, Anhui Zhenghua Biological Equipment Co., China) as described previously (*9*). Each rat was immobilized in a cylindrical immobilization device, and a mark was made 10 cm from the tip of the tail. The marked portion of the tail was placed on a platform where a plastic wedge applied pressure to the surface of the tail. The test was stopped and the value was recorded as the mechanical nociceptive threshold (MNT) if the rat flicked its tail, screamed, or struggled. A cutoff pressure of 600 g was used to avoid tissue damage.

2.4.2. *Paw withdrawal test*

Thermal nociception was measured with a plantar test apparatus (IITC, USA) as described previously (*10*). Each rat was put in a plexiglass box on a glass plate. A beam of light (30 W, 5 mm) passed through the glass to the sole near the toes both ipsilateral and contralateral to the surgical site. The paw withdrawal latency (PWL) was recorded as the time from the projection of the light beam to paw withdrawal. A cut-off time of 20 seconds was used to avoid tissue damage.

2.5. *Enzyme-linked Immunosorbent Assay (ELISA)*

The lumbar spinal cord (L3-L6) and dorsal root ganglia (L3-L6) were collected from each rat in a 2-mL EP tube filled with iced PBS (weight/volume ratio: 1 mg/100 uL). After the sample was homogenized and centrifuged, the supernatants were removed for immediate use. The amounts of IL-1β, TNF-α, and IL-6 were assayed using commercially available rat-specific ELISA kits (Shanghai Yikesai Biological Products Co., Shanghai, China) in accordance with the manufacturer's instructions as described previously (Ref. PMID: 27195494).

2.6. *Statistical analysis*

All data are presented as the mean±standard deviation (SD). SPSS for Windows 20.0 (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis. Changes in continuous variables over time were analyzed with a repeated measures analysis of variance, followed by Fisher's protected least significant difference (PLSD) test. Differences among groups at each time point and differences in each group at multiple time points were analyzed using an analysis of variance and the PLSD test. A paired samples *t* test was used for comparison (incised ipsilateral site *vs*. contralateral site). $p < 0.05$ was considered statistically significant. A scatter diagram was drawn before statistical analysis to exclude apparent outliers.

3. Results

3.1. *LIA and SNB partly prevented mechanical and thermal hyperalgesia induced by fentanyl and a plantar incision*

Changes in MNTs are shown in Figure 2A. The MNTs

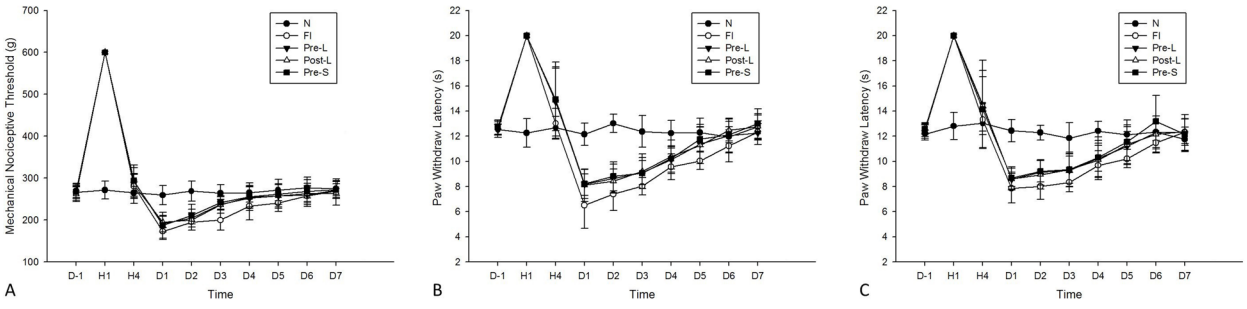

Figure 2. Local infiltration analgesia (LIA) and sciatic nerve block (SNB) partly prevent mechanical and thermal hyperalgesia induced by fentanyl and a plantar incision. SD rats in the normal group received no intervention. The other rats received subcutaneous fentanyl (60 μg/kg*4) and a plantar incision with or without local anesthesia with levobupivacaine (0.5%, 0.2 mL). (N group = normal rats; FI group = rats receiving fentanyl and an incision; Pre-L group = rats receiving pre-incision LIA, Post-L group = rats receiving post-incision LIA, and Pre-S group = rats receiving pre-incision SNB). The mechanical nociceptive threshold (g) (A) and paw withdrawal latency (s) of incised paws (B) and contralateral paws (C) on the day before drug injection (D-1), 1 and 4 hours after injection, and 1, 2, 3, 4, 5, 6, and 7 days later (D1-D7) are presented as the mean ± SD ($n = 8$).

increased significantly after 1 hour in the FI and Post-L groups and after 1 and 4 hours in the Pre-L and Pre-S groups, and the MNTs decreased significantly on days 1-5 after fentanyl injections. The decrease in MNTs in the FI group continued for 5 days (D1 to D5) and the decrease in MNTs in the Pre-L, Post-L, and Pre-S groups continued for 3 days (D1 to D3) compared to baseline values and MNTs in the normal group. In addition, the MNTs in the FI group were significantly lower than those in the Pre-L group on D3, in the Post-L group on days 1 and 3, and in the Pre-S group on days 3 and 4 ($p < 0.05$ according to a PLSD test).

The changes in thermal nociceptive latencies (shown as PWLs) ipsilateral to the surgical site are shown in Figure 2B. Similar to MNTs, the PWLs increased significantly after 1 hour in the FI group and after 1 and 4 hours in the Pre-L, Post-L, and Pre-S groups, and the PWLs decreased significantly on days 1-6 after fentanyl injections. The decrease in PWLs continued for 6 days (D1 to D6) in the FI group, for 4 days (D1 to D4) in the Pre-L and Pre-S groups, and for 5 days (D1 to D5) in the Post-L group compared to baseline values and PWLs in the normal group. In addition, the PWLs in the FI group were significantly lower than those in Pre L on days 1, 2 and 5, in the Post-L group on days 1, 3, 5 and 6, in the Pre-S group on days 1, 2 and 5 ($p < 0.05$ according to a PLSD test).

Interestingly, the changes in PWLs at sites contralateral to the surgical site (Figure 2C) were similar to those at ipsilateral sites (Figure 2B). Injections of fentanyl produced significant early thermal analgesia and subsequently produced significant hyperalgesia on days 1-5 in the FI, Pre-L, Post-L, and Pre-S groups. However, the thermal hyperalgesia exhibited at contralateral sites lasted less time than that at incision sites in the FI group (5 days *vs*. 6 days) and in the Post-L group (4 days *vs*. 5 days). Moreover, the PWLs at ipsilateral sites were significantly lower than those at contralateral sites on days 1, 2 and 3 in the FI group and on days 1 and 2 in the Pre-L, Post-L, and Pre-S groups after surgery ($p < 0.05$ for all according to a paired-samples *t* test).

No statistically significant differences in MNTs and PWLs were noted among the Pre-L, Post-L, and Pre-S groups.

In brief, the above results suggested that LIA and SNB partly prevented mechanical and thermal hyperalgesia induced by fentanyl and an incision.

3.2. *LIA and SNB partly prevented an increase in pro-inflammatory cytokines in the spinal cord and dorsal root ganglia induced by fentanyl and a plantar incision*

Levels of IL-1β (Figure 3), IL-6 (Figure 4), and TNF-α (Figure 5) expression in the spinal cord and dorsal root ganglia were determined 1 day before fentanyl injections, 4 hours after injections, and 1, 3, 5, and 7 days afterwards.

In the normal group, the expression of IL-1β, IL-6 and TNF-α did not significantly change over time. However, the level of IL-1β significantly increased in the spinal cord and dorsal root ganglia in the FI, Pre-L, Post-L, and Pre-S groups 1, 3, 5, and 7 days after fentanyl injections compared to baseline values and the levels of IL-1β in the normal group ($p < 0.05$ for all according to a PLSD test). Moreover, the levels of IL-1β in the FI group were significantly higher than those in the Pre-L group on days 1 and 3, in the Post-L group on days 1, 3, and 5, and in the Pre-S group on days 1, 3, 5, and 7 after fentanyl injections in the spinal cord ($p < 0.05$ according to a PLSD test) (Figure 3A). The levels of IL-1β in the FI group were significantly higher than those in the Pre-L and Pre-S groups on days 5 and 7, and in the Post-L group on day 5 after fentanyl injections in the ipsilateral dorsal root ganglion (DRG) ($p < 0.05$ for all according to a PLSD test) (Figure 3B). Similarly, the levels of IL-1β in the FI group were significantly higher than those in the Pre-L and Pre-S groups on day 5 and in the Post-L group on days 1 and 7 after fentanyl injections in the contralateral DRG ($p < 0.05$ according to a PLSD test) (Figure 3C). However, the levels of IL-1β in the ipsilateral DRG were significantly higher than those in the contralateral DRG in the FI and Pre-L groups 4 hours after injection and 1, 3, 5, and 7 days later, in the Post-L group 4 hours after injection and 1 and 3 days later, and in the Pre-S group 4 hours after injection and 1, 3, and 5 days later ($p < 0.05$ according to a paired samples *t* test).

Similarly, the levels of IL-6 significantly increased in the FI, Pre-L, Post-L, and Pre-S groups on days 1, 3, 5, and 7 in the spinal cord, in the FI and Pre-S groups on days 1, 3, 5, and 7, and in the Pre-L and Post-L groups on days 3, 5, and 7 in the dorsal root ganglia after fentanyl injections compared to baseline values and the levels of IL-6 in the normal group ($p < 0.05$ according to a PLSD test). Moreover, the levels of IL-6 in the FI group were significantly higher than those in the Pre-L and Post-L groups on days 1, 3 and 5 and in the Pre-S group on days 1, 3, 5, and 7 after fentanyl injections in the spinal cord ($p < 0.05$ according to a PLSD test) (Figure 4A). The levels of IL-6 in the FI group were significantly higher than those in the Pre-L and Post-L groups on days 5 and 7 and in the Pre-S group on days 3, 5, and 7 after fentanyl injections in the ipsilateral DRG ($p < 0.05$ according to a PLSD test) (Figure 4B). The levels of IL-6 in the FI group were significantly higher than those in the Pre-L group on days 3, 5, and 7, in the Post-L group on days 5 and 7, and in the Pre-S group on day 5 after fentanyl injections in the contralateral DRG ($p < 0.05$ according to a PLSD test) (Figure 4C). However, the levels of IL-6 in the ipsilateral DRG were significantly lower than those in the contralateral DRG on days 5 and 7 in the Pre-L group and on day 5 in the Post-L group ($p < 0.05$ according to a paired samples *t* test).

Similarly, the levels of TNF-α significantly

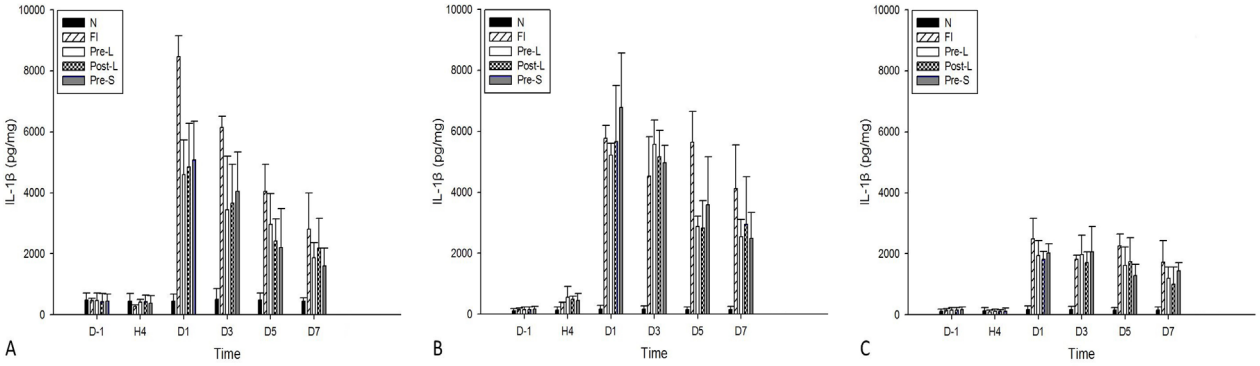

Figure 3. Local infiltration analgesia (LIA) and sciatic nerve block (SNB) partly prevent an increase in interleukin 1β (IL-1β) in the spinal cord induced by fentanyl and a plantar incision. SD rats in the normal group received no intervention. The other rats received subcutaneous fentanyl (60 μg/kg*4) and a plantar incision with or without local anesthesia with levobupivacaine (0.5%, 0.2 mL). (N group = normal rats; FI group = rats receiving fentanyl and an incision; Pre-L group = rats receiving pre-incision LIA, Post-L group = rats receiving post-incision LIA, and Pre-S group = rats receiving pre-incision SNB). The lumbar spinal cord **(A)** and the ipsilateral DRG **(B)** and contralateral DRG **(C)** were collected from surgical sites in 4 rats from each group. The expression of IL-1β (pg/mg) on the day before drug injection (D-1), 4 hours after injection (H4), and 1, 3, 5, and 7 days later (D1, D3, D5, and D7) was detected with an enzyme-linked immunosorbent assay (ELISA). All data are presented as the mean ± SD (*n* = 4).

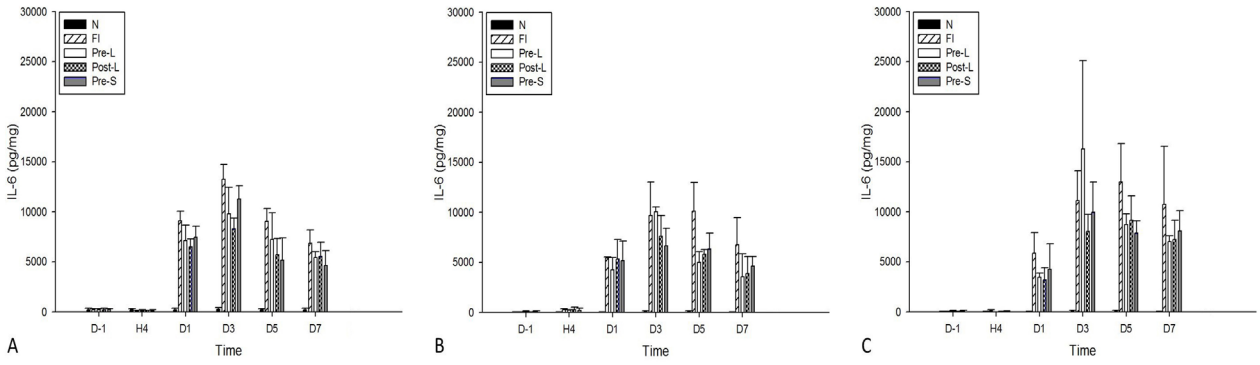

Figure 4. Local infiltration analgesia (LIA) and sciatic nerve block (SNB) partly prevent an increase in interleukin 6 (IL-6) in the spinal cord and dorsal root ganglia induced by fentanyl and a plantar incision. SD rats in the normal group received no intervention. The other rats received subcutaneous fentanyl (60μg/kg*4) and a plantar incision with or without local anesthesia with levobupivacaine (0.5%, 0.2 mL). (N group = normal rats; FI group = rats receiving fentanyl and an incision; Pre-L group = rats receiving pre-incision LIA, Post-L group = rats receiving post-incision LIA, and Pre-S group = rats receiving pre-incision SNB). The lumbar spinal cord **(A)** and the ipsilateral DRG **(B)** and contralateral DRG **(C)** were collected from surgical sites in 4 rats from each group. The expression of IL-6 (pg/mg) on the day before drug injection (D-1), 4 hours after injection (H4), and 1, 3, 5, and 7 days later (D1, D3, D5, and D7) was detected with an enzyme-linked immunosorbent assay (ELISA). All data are presented as the mean ± SD (*n* = 4).

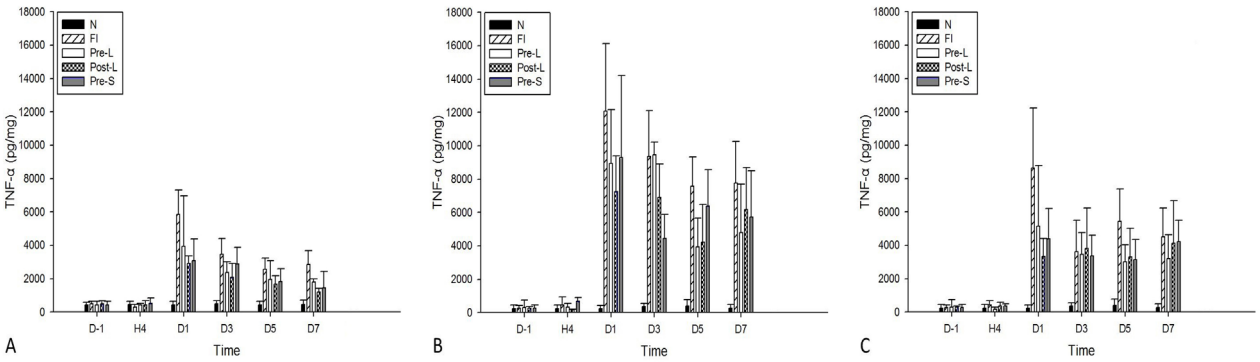

Figure 5. Local infiltration analgesia (LIA) and sciatic nerve block (SNB) partly prevent an increase in tumor necrosis factor α (TNF-α) in the spinal cord and dorsal root ganglia induced by fentanyl and a plantar incision. SD rats in the normal group received no intervention. The other rats received subcutaneous fentanyl (60 μg/kg*4) and plantar incision with or without local anesthesia with levobupivacaine (0.5%, 0.2 mL). (the N group = normal rats; FI group = rats receiving fentanyl and an incision; the Pre-L group = rats receiving pre-incision LIA, the Post-L group = rats receiving post-incision LIA, the Pre-S group = rats receiving pre-incision SNB). The lumbar spinal cord **(A)** and the ipsilateral DRG **(B)** and contralateral DRG **(C)** were collected from surgical sites in 4 rats from each group. The expression of TNF-α (pg/mg) on the day before drug injection (D-1), 4 hours after injection (H4), and 1, 3, 5, and 7 days later (D1, D3, D5, and D7) was detected with an enzyme-linked immunosorbent assay (ELISA). All data are presented as the mean ± SD (*n* = 4).

increased in the FI and Pre-L groups on days 1, 3, 5, and 7 in the spinal cord and dorsal root ganglia, in the Post-L and Pre-S groups on days 1, 3 and 5 in the spinal cord, and in the Post-L and Pre-S groups on days 1, 3, 5, and 7 in the dorsal root ganglia after fentanyl injections compared to baseline values and the levels of TNF-α in the normal group ($p < 0.05$ for all according to a PLSD test). Moreover, the levels of TNF-α in the FI group were significantly higher than those in the Pre-L group on day 1, in the Post-L group on days 1, 3 and 7, and in the Pre-S group on days 1 and 7 after fentanyl injections in the spinal cord ($p < 0.05$ according to a PLSD test) (Figure 5A). The levels of TNF-α in the FI group were significantly higher than those in the Pre-L group on days 1, 5, and 7, in the Post-L group on days 1 and 5, and in the Pre-S group on day 3 after fentanyl injections in the ipsilateral DRG ($p < 0.05$ for all according to a PLSD test) (Figure 5B). The levels of TNF-α in the FI group were significantly higher than those in the Pre-L, Post-L, and Pre-S groups on days 1 and 5 after fentanyl injections in the contralateral DRG ($p < 0.05$ according to a PLSD test) (Figure 5C). However, the levels of TNF-α in the ipsilateral DRG were significantly higher than those in the contralateral DRG on day 3 in the FI and Pre-L groups and on day 1 in the Post-L group ($p < 0.05$ according to a paired samples *t* test).

In brief, the above results indicated that local anesthesia partly prevented an increase in IL-1β, IL-6, and TNF-α in the spinal cord and dorsal root ganglia induced by fentanyl and a plantar incision. Fentanyl and an incision induced a greater increase in IL-1β and TNF-α but a lower increase in IL-6 in the ipsilateral DRG than in the contralateral DRG.

4. Discussion

This study found that an incision and fentanyl induced mechanical and thermal nociceptive hyperalgesia and increases in the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in the spinal cord and dorsal root ganglia ipsilateral and contralateral to the surgical site in rats. Local anesthesia with levobupivacaine partly prevented perioperative fentanyl-induced hyperalgesia and up-regulation of pro-inflammatory cytokines in the spinal cord and dorsal root ganglia. Three forms of local anesthesia - pre-incision LIA, pre-incision SNB, and postoperative LIA - had a similar effect on postoperative pain.

A previous study by the current authors (*11*) and another study (*12*) found that opioids such as fentanyl induce significant hyperalgesia, especially when a high dose has been administered. This phenomenon is called opioid-induced hyperalgesia (OIH). Although the detailed mechanism of this phenomenon is still unclear, it is presumably associated with activation of the N-methyl-D-aspartate (NMDA) receptor, reduced facilitation mediated by on- and off- cells within the rostro-ventral medulla (RVM), up-regulation of spinal dynorphin, an increase in excitatory peptide neurotransmitters such as cholecystokinin (CCK), and activation of the transient receptor potential vanilloid receptor (TRPV1) (*13*). Neuroimmune and neuroinflammatory interactions in the central nervous system (CNS) have also been found to contribute to the development of OIH (*11,14*). A previous study by the current authors found that repeated injections of high doses of fentanyl (60 μg/kg*4) induced a significant decrease in the mechanical and thermal threshold, activation of microglia in the spinal cord, and increased pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 in the spinal cord and DRG (*11*).

A surgical incision and trauma usually induce postoperative mechanical allodynia and thermal hyperalgesia in human and animal models (*15,16*). Interestingly, a previous study by the current authors (*11*) found that intraoperative administration of high-dose fentanyl resulted in greater hyperalgesia than a surgical incision only, as other studies had found (*17,18*). These findings suggest that perioperative opioids aggravate surgery-induced postoperative hyperalgesia, and especially at high doses; this finding is consistent with the results of several clinical trials (*3,13*).

The current study investigated mechanical nociception in the tail and thermal nociception at sites ipsilateral and contralateral to a surgical incision, unlike most studies in which the authors assessed only hyperalgesia at the surgical site (*17,18*). Surprisingly, mechanical hyperalgesia in the tail and thermal hyperalgesia at contralateral sites were significant and obvious. To be fair, thermal hyperalgesia at contralateral sites was less severe than that at ipsilateral sites early after surgery (Figures 2B and 2C). Since a tail pressure test reflects spinal reflexes involving both sides of the spinal cord (*19*) and since significant hyperalgesia was noted on the contralateral side, the current findings suggest that central sensitization may largely be involved in the development of delayed hyperalgesia induced by surgery and intraoperative fentanyl.

The current study also investigated pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in the spinal cord and dorsal root ganglia. Results indicated that they significantly increased in the spinal cord and dorsal root ganglia, following a surgical intervention and intraoperative administration of fentanyl with or without local anesthesia. The conjecture is that the mechanism of central sensitization mainly contributed to the hyperalgesia induced by surgery with fentanyl. However, the up-regulation of pro-inflammatory cytokines in the dorsal root ganglia suggests that a peripheral mechanism has also contributed to hyperalgesia to some extent. As an example, a greater increase in IL-1β and TNF-α in the DRG ipsilateral to the surgical site than in the contralateral DRG may explain why thermal hyperalgesia at ipsilateral sites was more severe than that

at contralateral sites.

Local anesthesia, including LIA and regional nerve block, has been found to prevent the development of postoperative pain at least during the acute postoperative period (*4,20,21*). The inhibitory effect on postoperative pain by local anesthesia may be mediated by preventing the transmission of nociceptive stimuli from the peripheral to the central nervous system. Clinically, opioids are commonly used during surgical procedures. Therefore, patients are usually given opioids for local anesthesia. The question is whether local anesthesia can prevent hyperalgesia induced by both a surgical incision and an opioid. The current study found that three forms of local anesthesia (including pre- and post-operative LIA and SNB) partly but clearly relieved mechanical hyperalgesia in the tail and thermal hyperalgesia at sites ipsilateral and contralateral to a surgical incision as a result of a plantar incision and perioperative fentanyl. These results corroborate the contention of Rivat *et al*.(*22*) that regional anesthesia is able to reduce postoperative acute hyperalgesia and chronic pain induced by the surgery itself and intraoperative OIH (*22*). However, the finding disagrees with the results of Meleine *et al*., who found that SNB failed to prevent incisional pain when high-dose fentanyl was administered to rats (*23*). The reason for this inconsistency is unclear. Meleine *et al*. postulated that high doses of fentanyl for intraoperative analgesia induced central sensitization and that SNB could not prevent central sensitization (*23*). However, the current study found that local anesthesia partly prevented mechanical and thermal hyperalgesia distant from a surgical site, and this study noted an increase in pro-inflammatory cytokines in the spinal cord and ipsilateral DRG induced by an incision and fentanyl in rats (Figure 2A,C and Figure 3,4,5). This indicates that local anesthesia may at least partly prevent central sensitization. The mechanism by which local anesthesia inhibits hyperalgesia distant from a surgical site is unclear. A previous study found that changes in the excitability of sensory neurons in the DRG may generate central connections to the spinal cord and then activate the glia in the spinal cord (*24*). The activation of spinal glia and an increase in pro-inflammatory cytokines might induce a secondary increase in pro-inflammatory cytokines in the contralateral DRG. This is perhaps part of the reason why local infiltration anesthesia and a nerve block at the ipsilateral site ultimately had an effect on hyperalgesia distant from a surgical site.

Chronic pain is associated with long-term changes in levels of pro-inflammatory cytokines in the spinal cord and the excitability of sensory neurons in the DRG (*25*). These findings imply that uncontrolled and persistent activity of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α may induce a transition from acute pain to chronic pain. This suggests that the anti-inflammatory benefits of local anesthesia were a result of reducing short-term postoperative pain as well as preventing the development of chronic postoperative pain somewhat. In addition, sufficient local anesthesia can also facilitate a lower dosage of opioids perioperatively, which may reduce the incidence of OIH.

In the current study, pre-incision LIA and a pre-incision SNB had similar effectiveness in terms of anti-nociceptive and anti-inflammatory activity. Pre-incision LIA has the advantages of a simpler procedure and it causes fewer adverse events, so it is warranted for pain control. Moreover, pre-incision LIA and post-incision LIA differed little in terms of anti-nociceptive and anti-inflammatory activity, indicating that remedial local anesthesia is still valid.

The current study has several limitations. *i*) The dose of fentanyl given to rats was 240 μg/kg in total, which is equal to 38 μg/kg in humans after converting based on body surface area (*26*). This dose was much higher than the dose commonly used in clinical practice. High-dose fentanyl was used in the rat model because a previous study by the current authors noted behavioral hyperalgesia and spinal inflammation induced by fentanyl. The higher dose of fentanyl may cause more distinct behavior and neuroinflammation and allow those mechanisms to be more clearly understood. However, the clinical significance and relevance of this study diminished since such a high dose of fentanyl may cause numerous adverse effects, which is why it is not usually administered to patients undergoing surgery. Further preclinical studies using animal models need to examine surgery and clinical doses of fentanyl in order to obtaining clinically relevant data and to facilitate the transition from bench to bed. *ii*) This study used an animal model combining a surgical incision with intraoperative fentanyl. This approach may be similar to clinical practice and provide useful data for clinical reference since opioids are indispensable during surgery. However, this approach incorporates the effect of local anesthesia on hyperalgesia and neuroinflammation, and it does not allow a distinction between fentanyl-induced hyperalgesia and post-incision hyperalgesia since both conditions can induce hyperalgesia.

In conclusion, the current study noted increased expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in the spinal cord and dorsal root ganglia in a rat model of a plantar incision with intraoperative fentanyl. Pre-incision LIA, a pre-incision SNB, or post-incision LIA can partly prevent hyperalgesia and up-regulation of pro-inflammatory cytokines in the spinal cord and dorsal root ganglia induced by surgery and fentanyl.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (project no. 81571071) and

the Guangdong Provincial Natural Science Foundation of China (project no. 2014A030313203).

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(Received January 30, 2018; Revised March 11, 2018; Accepted March 27, 2018)