ORIGINAL RESEARCH

Sciatic Nerve Block Combined with Flurbiprofen Inhibits Spinal Cord Inflammation and Improves Postoperative Pain in Rats with Plantar Incision

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Background and Purpose: Peripheral nerve block is often used to relieve postoperative pain. But the effect of nerve block on inflammatory response is not fully understood. Spinal cord is the primary center of pain processing. This study is to investigate the effect of single sciatic nerve block on the inflammatory response of the spinal cord in rats with plantar incision and the combined effect with flurbiprofen.

Methods: The plantar incision was used to establish a postoperative pain model. Single sciatic nerve block, intravenous flurbiprofen or the combination of both were used for intervention. The sensory and motor functions after nerve block and incision were evaluated. The changes of IL-1β, IL-6, TNF-α, microglia and astrocytes in the spinal cord were examined by qPCR and immunofluorescence respectively.

Results: Sciatic nerve block with 0.5% ropivacaine in rats induced sensory block for 2h and motor block for 1.5h. In the rats with plantar incision, the single sciatic nerve block did not alleviate postoperative pain or inhibit the activation of spinal microglia and astrocytes, but the levels of IL-1β and IL-6 in spinal cord were decreased when the nerve block wore off. The combined effect of a single sciatic nerve block and intravenous flurbiprofen not only decreased the levels of IL-1β, IL-6, and TNF-α, but also relieved the pain and alleviated the activation of microglia and astrocytes.

Conclusion: The single sciatic nerve block cannot improve postoperative pain or inhibit the activation of spinal cord glial cells, but can reduce the expression of spinal inflammatory factors. Nerve block combined with flurbiprofen can inhibit spinal cord inflammation and improve postoperative pain. This study provides a reference for rational clinical application of nerve block.

Keywords: sciatic nerve block, inflammatory response, spinal cord, flurbiprofen, plantar incision, postoperative pain

Introduction

More than 313 million surgeries are performed worldwide each year. 49.5% of them suffer from moderate pain after surgery,[1] and 20% of them suffer from severe pain.[2] If the postoperative pain is not managed properly, it will cause respiratory and circulatory complications, affect postoperative rehabilitation, delay discharge time and increase the risk of wound infection.[3] Severe acute pain can even turn into chronic pain,[4,5] which affects sleep, mood, daily activities, and reduces the patient's quality of life.[6,7] Therefore, prevention and management of postoperative pain are very important.

In the past 20 years, the development of ultrasound visualization technology has made the clinical application of nerve block more common.[8–10] Compared with general anesthesia alone, nerve block can relieve postoperative pain, reduce postoperative opioid requirement, and promote early recovery of patients.[11–13] However, some patients suffered from severe pain at 8–24h after the nerve block wore off. This phenomenon is defined as "rebound pain" or "breakthrough pain". The incidence of rebound pain in orthopedic surgery is as high as 52%,[14–19] and this phenomenon may

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even offset the overall benefit of nerve block to patients. Thus, the effect of preoperative single nerve block on postoperative pain was not definitely determined.

It is known that the development of acute and chronic pain is associated with neuroinflammation.[20,21] However, the effect of nerve block on inflammatory response still remains controversial. Beloeil et al used bupivacaine to block the sciatic nerve of rats and found that it alleviated carrageenin-induced inflammation in the hindpaw,[22] but other studies have shown that nerve block has a pro-inflammatory effect and can facilitate inflammation around the wound.[23,24] Clinical studies have found that, compared with general anesthesia, general anesthesia combined with thoracic para-vertebral nerve block can reduce the systemic inflammatory response and the level of inflammatory factors in thoracic surgical patients.[25,26] Zhen et al indicated that nerve block significantly alleviated clinical inflammatory manifestations, such as the reduction of knee circumference and skin temperature, but inflammatory factors in tissues and plasma were not decreased.[27] However, these studies mostly focused on the changes in local and systemic inflammatory cytokines. In addition, spinal cord is the primary center of pain processing, the effect of nerve block on the inflammatory response of the spinal cord in postoperative rats was not fully understood.

With the development of enhanced recovery after surgery (ERAS), multimodal analgesia has been widely advocated in perioperative pain management, which makes patients to obtain better analgesic effect and lower complications.[28] Multimodal analgesia refers to combining different methods or drugs to control pain.[29,30] At present, nerve block based analgesia program with low opioid analgesic regimens are recommended. Nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase (COX), and can play a powerful analgesic action on the incision and inflammation tissue with high affinity, which can relieve various postoperative pain. Flurbiprofen is a commonly used auxiliary medication and it can inhibit both fatty acid amide hydrolase and COX, which may be a potential useful analgesic.[31,32] Preoperative or intraoperative intravenous application of flurbiprofen can effectively inhibit proinflammatory cytokines, and reduce postoperative pain.[33]

In this study, we first established the rat model with sciatic nerve block and evaluated the sensory and motor block time; then to investigate the effects of the single sciatic nerve block on postoperative pain, and the expression of glial cells and cytokines in the spinal cord in rats with plantar incision; last, to investigate the combinational effects of the nerve block with intravenous flurbiprofen.

Methods

Animals

Male SD rats (250–300g) were purchased from Tongji Medical College, Huazhong University of Science and Technology. All SD rats were housed in an SPF barrier environment with a temperature of 22–25°C, relative humidity of 45–65%, a light–dark cycle of 12h, and free eating and drinking. The experiments were carried out according to the Guide for the Use of Laboratory Animals and the National Institutes of Health Guide for the Care and approved by the Laboratory Animal Management Committee of Tongji Medical College, Huazhong University of Science and Technology (TJH-202201037).

Grouping

Seven days before the operation, SD rats were adapted to the test environment, and the basal pain threshold and motor function of rats were measured one day before the operation.

In experiment 1, SD rats were divided into two groups: ropivacaine block group (RP group, n = 8) and saline block group (NS group, n = 8).

In experiment 2: SD rats were divided into 4 groups: ropivacaine block plus plantar incision pain group (RP+PI group, n = 32) and normal saline plus plantar incision pain group (NS+PI group, n = 32); intravenous flurbiprofen plus saline block plus plantar incision pain group (F+NS+PI group, n = 32) and intravenous flurbiprofen plus ropivacaine block plus plantar incision pain group (F+RP+PI group, n = 32).

Animals that failed nerve block or died from anesthesia were excluded.

Sciatic Nerve Block

The model of the left sciatic nerve block was performed as previously described.[34,35] Briefly, after inhalational anesthesia with 2.5% isoflurane, the greater trochanter and the sciatic tuberosity of rats were touched, and a longitudinal incision was made along the posterolateral thigh of rats. The sciatic nerve was exposed after bluntly separating the biceps femoris muscle and semitendinosus muscle. 0.2mL of 0.5% ropivacaine or 0.9% normal saline was injected into the perineural fascia. After injection, the incision was sutured, and the surgical wound was sprayed with penicillin to prevent infection.

Plantar Incision

Under the effect of inhalation of 2.5% isoflurane anesthesia, after the sciatic nerve was blocked, the left plantar incision was performed as previously described.[36,37] A longitudinal incision of about 1cm in length was made from 0.5cm proximal plantar to the toe through the skin and fascia of the left paw including the underlying muscle. The plantar muscle was lifted with eye forceps and incised longitudinally and then put it back to the original position with the muscles' origin and insertion intact. After applying pressure to stop the bleeding, the incision was sutured. Erythromycin ointment was applied to the incision to prevent infection.

Flurbiprofen Administration

After sciatic nerve block and plantar incision, the rat tail was wiped with 75% alcohol and warm water for tail venipuncture. Normal saline or flurbiprofen (3E02IT, Beijing Teide Pharmaceutical Co., LTD.) was administered I. V. with 10mg/kg.

Behavioral Test

Assessment of Sensory Function

(a) Paw mechanical withdrawal threshold (PMWT): As described previously,[38,39] a set of von Frey filaments (Stoelting, Wood Dale, IL, USA) were used to evaluate PMWT by stimulating the middle area of the left hindpaw close to the incision. The rats were put in the plastic box to adapt for 30min, then different von Frey filaments ranging from 2 to 15g were used to stimulate the plantar vertically for at least 3 seconds. Positive reactions were considered if the rats suddenly shrank, shook or licked their paws, and positive reactions were detected by lower von Frey filaments. PMWT was the minimum force required to cause at least three positive reactions in five tests.

(b) Paw withdrawal Latency (PWL): A thermal pain stimulator (equipment model 37,370, Italy) was used to measure the nociceptive thermal stimulation. The rats were placed in a plastic box for 10–15min before the test, and the infrared light source was moved to the middle area near the incision of the left hindpaw of the rats When the rats lifted the paw due to pain, the device automatically detected and stopped timing, and the time of paw contraction was displayed on the monitor with an accuracy of 0.1s. To avoid tissue damage, 30s was taken as the cut value, and the infrared intensity was adjusted so that the basic PWL of SD rats was 15–20s.[40,41]

(c) Cumulative pain score (CPS): the weight bearing of left hindpaw was observed. The rats were placed in a transparent plastic box with a grid pad made of metal wire at the bottom. Before the test, the rats were placed in a transparent plastic box for 20 minutes, and the observation was performed once every 5 minutes, each lasting 1 minute. The sum of 12 scores (range: 0–24) was obtained during the 1h testing period. The CPS was then obtained by subtracting the score for the injured paw from that for the non-injured paw. Scoring method: the hindpaw completely touches the wire, the hindpaw lands on the ground, and the weight gauge scores 0; the wound has deformation when touching the net, landing on the ground but not bearing meter 1 point; the sole does not touch the iron net completely, and the hindpaw does not raise the ground to count 2 points.[41,42]

Assessment of Motor Function

According to observing the toes' movement reflex (peroneal nerve motor fibers) to assess the motor function of rats, this is a kind of vestibular reflex, by lifting the rats to observe whether the reaction of the stretching and contraction, to record the rat movement recovery, and motor function was evaluated every 10 minutes until the block effect wore off. Scoring

method: no paw extension reflex and grasp count as 2 points; weak paw extension reflex recovery and grasp count as 1 point; total paw reflex and strong grasp count as 0 points.[24,43]

Immunofluorescent Staining

Rats were sacrificed under deep anesthesia before the operation and at 6h, 24h, 3d, 7d after nerve block. Four samples were taken from each group at each time point. The spinal cord tissue was removed rapidly and fixed in 4% paraformaldehyde at 4°C for 2d. Then the harvested spinal cord was dehydrated in a gradient and sectioned to 20μm thickness in a cryostat (CM, Leica, Germany). The sections were blocked with 5% donkey serum or goat serum for 1 hour at room temperature. The microglial marker Iba1 (AB5076, 1:200, Abcam) and the astrocyte marker GFAP (#3670, 1:200, Cell Signaling Technology) were incubated overnight. Then, donkey anti-goat secondary antibody labeled with Alexa Fluor 488-immune research (Jackson ImmunoResearch, 1:100, West Grove, PA, USA) and Alexa Fluor 488-labeled goat anti-mouse secondary antibody (Jackson ImmunoResearch, 1:200, West Grove, PA, USA) were incubated for 2 hours at room temperature. U-HGLGPS immunofluorescence microscope (Olympus, Japan) was used to obtain images and analyze the changes in the number of microglia. The expression number of microglia per unit area could be calculated by using the average value per 50μm×50μm.

qPCR

Rats were sacrificed under deep anesthesia. The spinal cord tissue was removed rapidly. Total RNA was extracted with Trizol (No.108–95-2, Takara), and 1μg RNA was reversely transcribed into cDNA. Quantitative RT-PCR was performed using PCR system (ABI 7900/Viia, Foster City, CA, USA). SYBR Green was used to detect the amplification of β-actin, IL-1β, IL-6, and TNF-α. The primer sequences are shown in Table 1. The reaction conditions were 95°C for 2min (initial denaturation), followed by 45 cycles each consisting of 95°C for 10s (denaturation) and 60°C for 30s (annealing extension). β-actin was used as the internal parameter to correct the expression of the target genes. The relative level of IL-1β, IL-6, and TNF-α mRNA was calculated using the comparative cycle threshold (CT) method ($2^{-\Delta\Delta CT}$).

Statistical Analysis

Graphpad8.0 software was used for statistical analysis. The motor block results were expressed as mean ± standard error of the mean (X±SEM); Other results were expressed as mean ± standard deviation (X±SD). One-way ANOVA followed by Dunnett's test was used to analyze the data of PWL, PWMT, CPS, motor block, qPCR, and immunofluorescence within groups. Two-way ANOVA followed by Bonferroni's test was used to analyze the data among groups. $P < 0.05$ was considered statistically significant.

Table 1 The Primer Sequences

Gene (Accession No)	Primer	Sequence	Product Length
β-actin (NM_031144.3)	Forward	TGACAGGATGCAGAAGGAGA	104bp
	Reverse	TAGAGCCACCAATCCACACA	
IL-1β (NM_031512.2)	Forward	GTGGGATGATGACGACC	151bp
	Reverse	CACTTGTTGGCTTATGTTCT	
IL-6 (NM_012589.2)	Forward	CAGCCACTGCCTTCCCTA	157bp
	Reverse	TTGCCATTGCACAACTCTTT	
TNF-α (NM_012675.3)	Forward	GTCTACTGAACTTCGGGGTGAT	146bp
	Reverse	TGCTACGACGTGGGCTACA	

Results

Sensory and Motor Effect of Sciatic Nerve Block with 0.5% Ropivacaine

At 0.5h, 1h, 1.5h, 2h, 2.5h, 3h, 4h, and 6h after nerve block, the PWL and motor block scores were detected to evaluate the sensory and motor effect of sciatic nerve block with 0.5% ropivacaine (Figure 1A). Compared with the NS group, the PWL of the hindpaw in the RP group increased from 0.5h after nerve block and lasted for 2h (Figure 1B; $P < 0.05$), and PWL decreased temporarily at 4h after the operation ($P < 0.05$). At 0.5h after sciatic nerve block, the paw extension reflex of SD rats disappeared and dragged the floor and the duration of motor block was about 1.5h (Figure 1C; $P < 0.05$). These results indicated that ropivacaine blocked the rat sciatic nerve successfully.

Effect of Sciatic Nerve Block Combined with Flurbiprofen on Postoperative Pain and Spinal Cord Inflammation in Rats

Pain Behavior

At 0.5h, 2h, 6h, 1d, 2d, 3d, 5d, and 7d after nerve block, PWL, PMWT, and CPS were used to evaluate the analgesic effect of sciatic nerve block, flurbiprofen and their combination (Figure 2A). In NS+PI group, PWL began to decrease at 0.5h ($P < 0.05$) and returned to baseline at 7d after operation. Compared with the NS+PI group, the PWL in RP+PI group neither increased nor decreased after 2h ($P > 0.05$). The PWL of the F+NS+PI group returned to baseline at 5d. Compared with RP+PI group, although analgesia of flurbiprofen was not as effective as sciatic nerve block in the first 2h, it was superior to sciatic nerve block after 2h ($P < 0.05$). Compared with the RP+PI and F+NS+PI groups, PWL was further prolonged in the F+RP+PI group (Figure 2B).

The trend of PWMT in the NS+PI group was consistent with that of PWL. The PWMT in RP+PI group returned to baseline at 5d, while the PWMT in F+NS+PI and F+RP+PI groups returned to baseline at 3d, which was earlier than that in NS+PI group. The inter-group comparison of PWMT was consistent with that of PWL (Figure 2C).
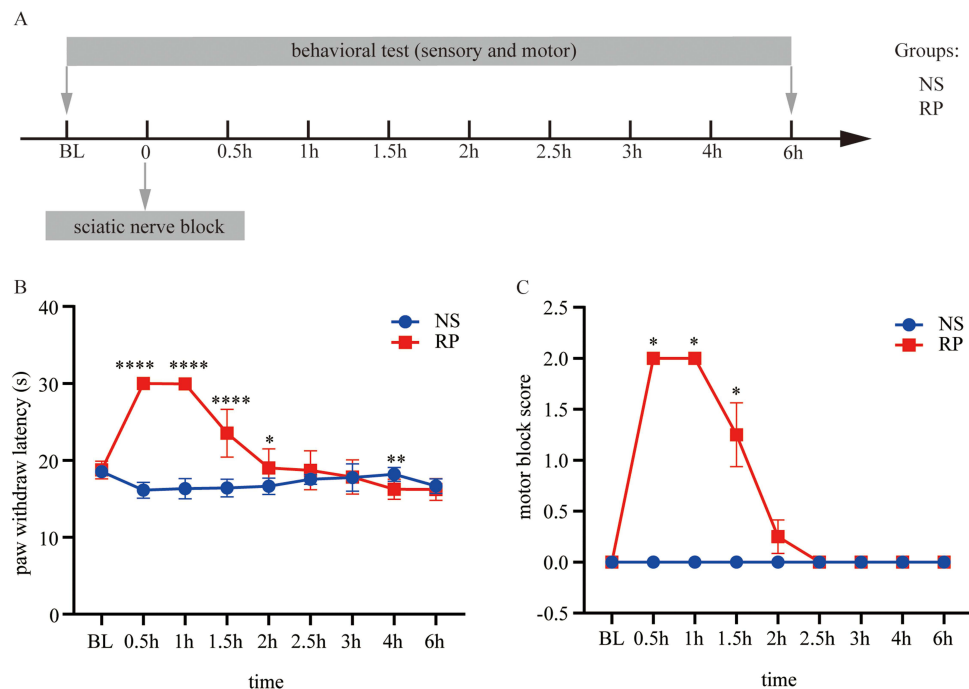
Figure 1 Effect of sciatic nerve block with 0.5% ropivacaine. (**A**) Experimental 1 designs and animal groups. (**B**) Paw withdrawal latency was assessed for 6h after sciatic nerve block. (**C**) Motor block scores were assessed by paw extension reflex for 6h after sciatic nerve block. The results of PWL are presented as the mean ± SD and the motor block scores are presented as the mean ± SEM (n = 8). Two-way ANOVA and Bonferroni's test were used to analyze the inter-group differences. (Compared with NS group, ****$P < 0.0001$, **$P < 0.005$, *$P < 0.05$).
Abbreviations: BL, baseline; NS, normal saline; RP, Ropivacaine.
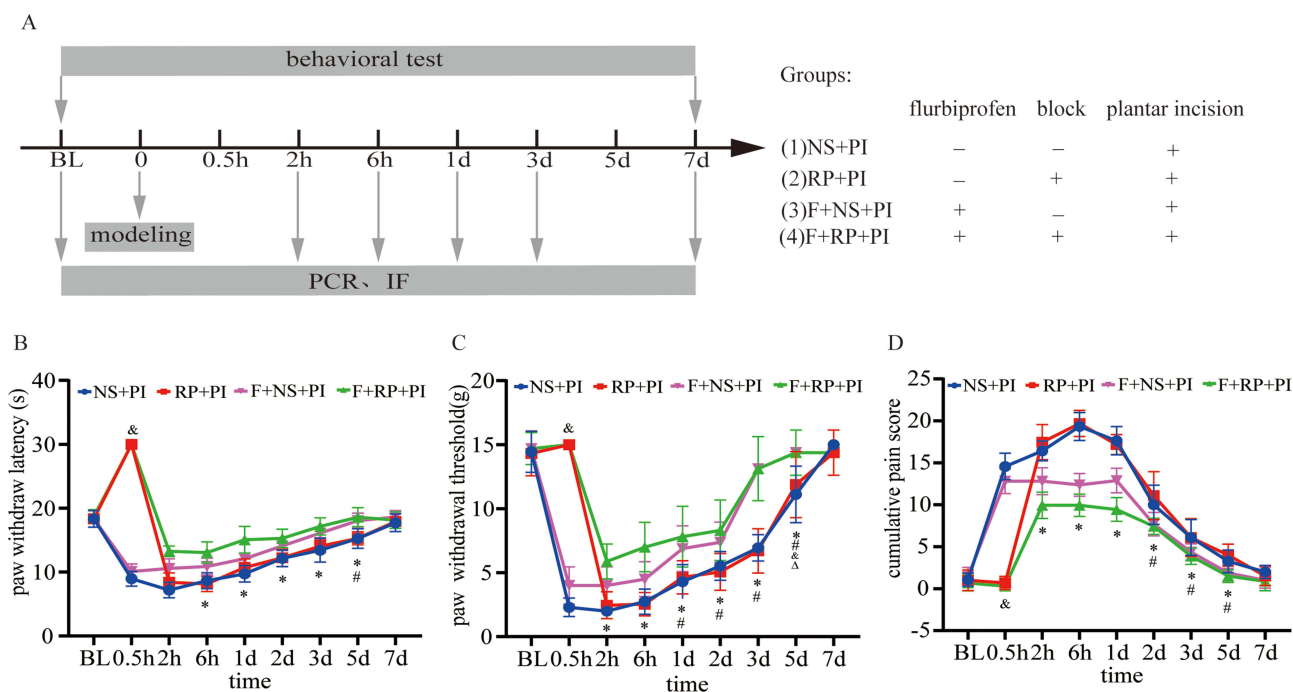
Figure 2 Effect of sciatic nerve block combined with flurbiprofen on postoperative pain in rats with plantar incision. (**A**) Experimental 2 designs and animal groups. (**B**) Paw withdrawal latency was assessed for 7d after sciatic nerve block or flurbiprofen injection in rats with incision. (**C**) Mechanical allodynia evaluated by the paw withdrawal threshold for 7d after sciatic nerve block or flurbiprofen injection in rats with incision. (**D**) Cumulative pain score was for 7d after sciatic nerve block or flurbiprofen injection in rats with incision. The results are presented as the mean ± SD (n = 8). Two-way ANOVA and Bonferroni's test were used to analyze the inter-group differences. (Compared with NS+PI group, RP+PI group *P >0.05; Compared with F+NS+PI group, F+RP+PI group #P >0.05; Compared with RP+PI group, F+RP+PI group &P >0.05, F+NS+PI group ΔP >0.05).

Abbreviations: BL, baseline; NS, normal saline; RP, Ropivacaine; F, Flurbiprofen; PI, plantar incision pain model.

In NS+PI group, CPS began to increase at 0.5h (P < 0.05), and returned to baseline at 7d after operation. CPS in RP+PI group began to increase at 2h and returned to baseline at 7d as in NS+PI group. CPS in F+NS+PI group also began to increase at 0.5h but returned to the baseline at 5d. However, CPS in F+NS+PI group was significantly decreased compared with NS+PI group (P < 0.05) and the combination of sciatic nerve block and flurbiprofen could further decrease CPS (Figure 2D). The above results indicated that the sciatic nerve block was less effective than intravenous flurbiprofen, but the combination of the two provided the best analgesia.

Changes in Spinal Glial Cells

Compared with baseline, microglia were mainly activated in the dorsal horn and medial side of the ipsilateral spinal cord at 1d after the operation in NS+PI and RP+PI groups (Figure 3A), which was earlier than those in F+NS+PI and F+RP+PI groups. Microglia were activated in all four groups at 3d after the operation, but the number of activated microglia in NS+PI and RP+PI groups was more than that in F+NS+PI and F+RP+PI groups (Figure 3B; P < 0.05).

Astrocytes in NS+PI and RP+PI groups were activated at 1d after the operation and astrocytes were mainly activated in the ipsilateral spinal dorsal horn (Figure 4A), while those in the F+NS+PI group were activated at 3d, which all lasted to 7d after the operation (Figure 4B; P < 0.05). Astrocytes in the F+RP+PI group were not activated at all time points.

Changes of Proinflammatory Factors in the Spinal Cord

Compared with the baseline, the level of IL-1β increased at 6h in NS+PI, RP+PI and F+NS+PI groups, while at 1d in F+RP+PI group (P < 0.05), and returned to the baseline at 7d in all groups (Figure 5A). The level of IL-1β in NS+PI group was significantly higher than that in the other three groups (P < 0.05). IL-6 increased only in the NS+PI group (P < 0.05), but not in the other groups (Figure 5B). The expression of TNF-α in the NS+PI, RP+PI and F+NS+PI groups also increased at 6h (P < 0.05). TNF-α lasted until 24h in F+NS+PI group and 3d in the other two groups, while there was no
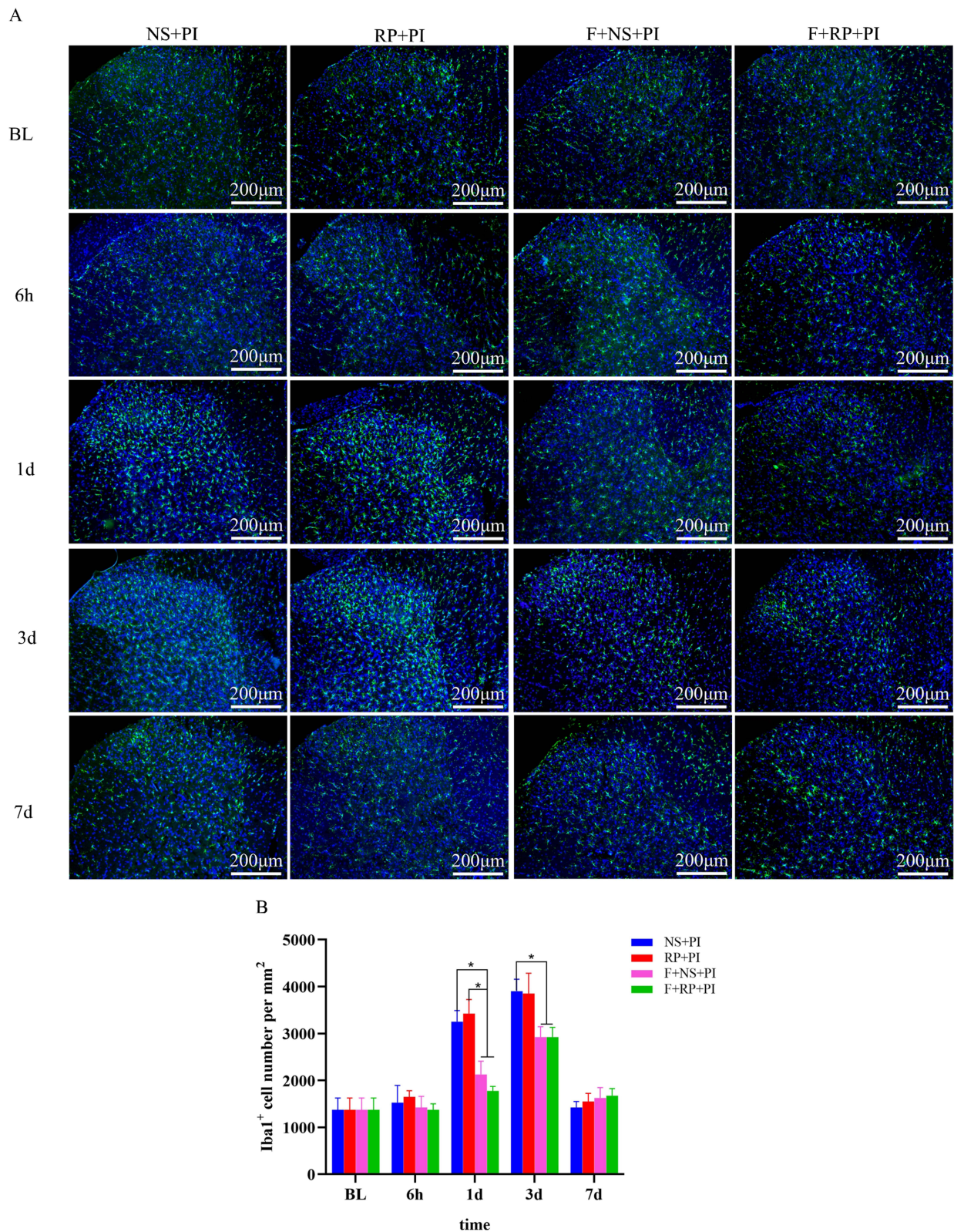
Figure 3 Activation of microglia in the spinal dorsal horn of rats with plantar incision. (**A**) Representative images of one rat from each group. (**B**) Quantification of the number of Iba1+ cells per square millimeter in the spinal cord horn (scale bar=200μm; Blue represents cell nucleus and green represents microglia). The results are presented as the mean ± SD (n = 4). Two-way ANOVA and Bonferroni's test were used to analyze the inter-group differences; *P < 0.05.
Abbreviations: BL, baseline; NS, normal saline; RP, Ropivacaine; F, Flurbiprofen; PI, plantar incision pain model.

A

B
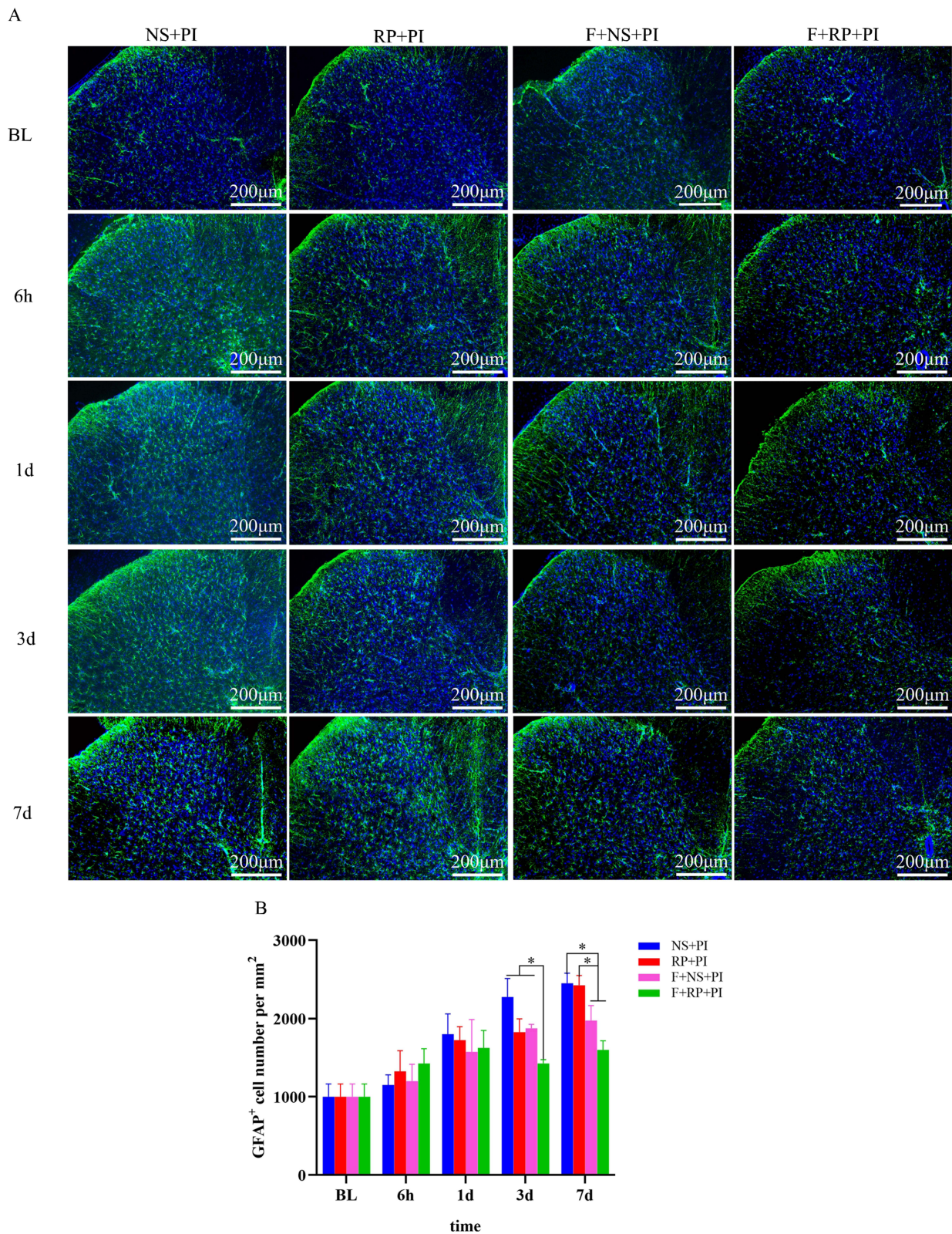
Figure 4 Activation of astrocytes in the dorsal horn of spinal cord in rats with plantar incision. (**A**) Representative images of one rat from each group. (**B**) Quantification of the number of GFAP+ cells per square millimeter in the spinal cord horn (scale bar=200μm; Blue represents cell nucleus and green represents astrocytes). The results are presented as the mean ± SD (n = 4). Two-way ANOVA and Bonferroni's test were used to analyze the inter-group differences;*P < 0.05.
Abbreviations: BL, baseline; NS, normal saline; RP, Ropivacaine; F, Flurbiprofen; PI, plantar incision pain model.
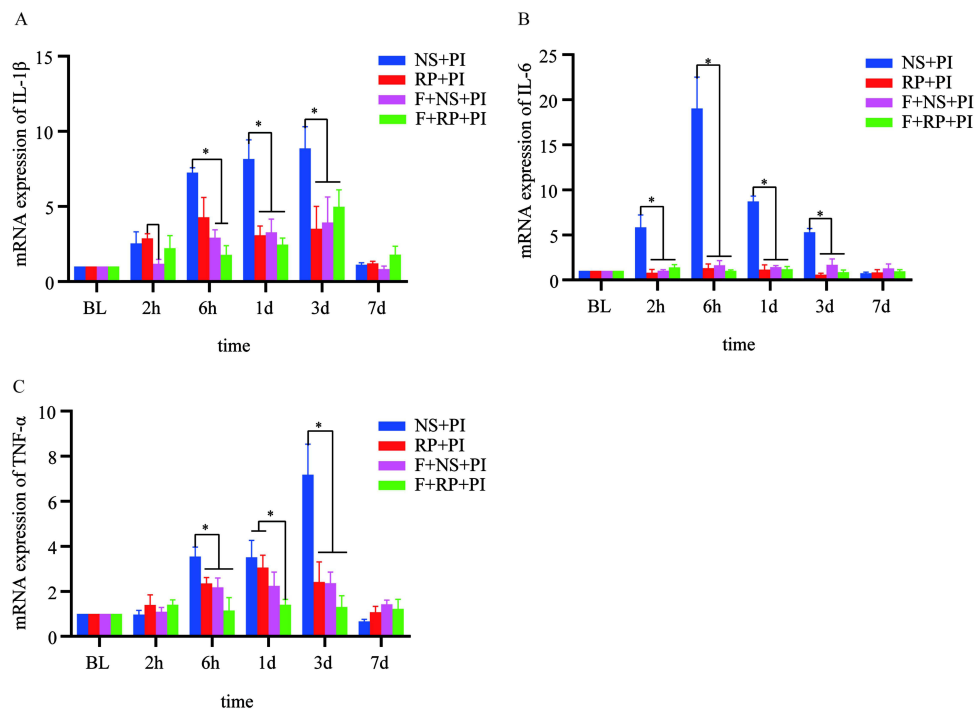
Figure 5 Changes of proinflammatory factors in the spinal dorsal horn of rats with plantar incision. (**A–C**) The expressions of IL-1β, IL-6 and TNF-α in the spinal dorsal horn. The results are presented as the mean ± SD (n = 4). Two-way ANOVA and Bonferroni's test were used to analyze the inter-group differences (comparison between two groups,*P<0.05).

change in the F+RP+PI group (Figure 5C). This indicates that sciatic nerve block or flurbiprofen can inhibit the expression of inflammatory factors, and the combined effect is better.

Discussion

In this study, we found that: (1) The single sciatic nerve block with 0.5% ropivacaine in rats induced about 2 hours of sensory block and 1.5 hours of motor block. (2) In rats with plantar incision, PWL and PMWT decreased and CPS increased at 0.5h and returned to baseline at 7d. Spinal inflammatory factors IL-1β, IL-6 and TNF-α increased significantly at 6h, and spinal microglia and astrocytes were activated at 24h. (3) Compared with the NS+PI group, the sciatic nerve block with 0.5% ropivacaine neither improved postoperative pain after 2h of operation when the block wore off, nor decrease the number of microglia and astrocytes in the spinal cord, but it decreased the expression of inflammatory factors. (4) Flurbiprofen could relieve the plantar incision pain. Although the analgesic effect was not as good as that of a single sciatic nerve block for the first 2h, the analgesic effect was better than that of a single sciatic nerve block after 2h, and it could relieve spinal cord inflammation. (5) The analgesic effect of a single sciatic nerve block combined with flurbiprofen was superior to that of the two alone, and could directly inhibit the activation of astrocytes.

Generally, the methods of sciatic nerve block in rats include direct nerve exposure,[34,35,44–46] anatomical localization,[47] or guided by nerve stimulator.[48,49] Anatomically located sciatic nerve block have the risk of incomplete block due to anatomic variation and low dose used. At present, the most commonly used method is direct nerve exposure. Although there is some trauma, direct exposure of the sciatic nerve is more accurate, and no significant changes in PWL, PWMT, and CPS of the ipsilateral paw occurred after the nerve block wears off.[35] After sciatic nerve block with 0.5% ropivacaine in rats, the PWL was prolonged, the left hindpaw was dragged to the ground, and the paw extension reflex was absent. The sensory block time was about 2h, and the motor block time was about 1.5h, which is consistent with previous studies.[45]

The animal models of acute pain include the injection of chemical inflammatory substances such as complete Freudenberg's adjuvant, carrageenan, and plantar incision.[41,50–52] The pain caused by chemical inflammatory substances

reached the peak at 6h and returned to normal at 3d. In the model of plantar incision, the pain threshold decreased at 1h after the operation, reached the peak at 24h, and returned to baseline at 7d, which was consistent with the trend of postoperative pain in clinical patients.[53,54] Therefore, the plantar incision model was used to simulate the patients' pain after surgery. The study found that the PWL and PWMT decreased significantly, and CPS increased at 0.5h in the NS+PI group, which returned to the baseline at 7d.

The spinal cord is the primary center of pain processing. Neuroinflammation is mediated by the activation of microglia and astrocytes, which plays an important role in the generation and maintenance of pain.[55,56] Previous studies have shown that the activation of spinal microglia and astrocytes and the release of inflammatory factors could be induced at 24h after plantar incision.[57,58] Studies have found that the up-regulation of p38 at 1h after plantar incision resulted in the release of brain-derived neurotrophic factor (BDNF) and pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, while the number of microglia was increased at 3d after operation.[59,60] Other studies have also found that the release of inflammatory factors from microglia was not consistent with its morphological changes.[61] Consistently, in our study, microglia and astrocytes were activated significantly at 24h after plantar incision. Microglia were mainly activated in the dorsal horn and medial side of the ipsilateral spinal cord, and astrocytes were mainly activated in the ipsilateral spinal dorsal horn. The levels of TNF-α, IL-1β, and IL-6 in the spinal cord significantly increased at 6h.

Our study found the single sciatic nerve block with 0.5% ropivacaine did not improve the postoperative pain after 2h of operation in rats with plantar incision, compared with the NS+PI group. It was consistent with the study of Yamada et al,[24] which may be due to the short block time. In addition, studies have shown that the sciatic nerve mainly innervated the lateral side of the paw, and the saphenous nerve mainly innervated the medial side of the paw.[62] In our study, the incision was made in the middle of the plantar area. It was possible that an incomplete nerve block led to nociceptive stimulus afferent to the spinal cord.

Rebound pain was manifested as sudden onset of severe pain within 8–24 hours after the nerve block, with numerical rating scale (NRS) ≥7. The pain lasted for 3 to 6 hours, and the nature of the pain was mostly burning, electric shock, needle-like or numbness. It could occur in a resting or moving state, and most occurred in orthopedic surgery.[14–16,63] Although rebound pain occurred in some patients after the nerve block wore off, it was not observed in this study. The possible reason was that the trauma caused by the plantar incision was not enough to induce rebound pain.

In Ding et al study, sciatic nerve block was performed before the plantar incision in neonatal mice, and then 3 times of sciatic nerve block was administered every 2 hours after the incision. They found multiple sciatic nerve block reduced the activation of microglia in the spinal dorsal horn and down-regulated BDNF and its downstream receptors, thereby alleviated neonatal incision-induced exaggravation of incision pain in adulthood.[64] This study found that although sciatic nerve block could reduce the levels of inflammatory factors in the spinal cord, it could not inhibit the activation of microglia and astrocytes, which may be due to the short duration of nerve block. In addition, Kroin et al also did not inhibit spinal cord inflammation in rats with plantar incision,[65] indicating that spinal cord inflammation was not entirely mediated by afferent nerve activity. Studies have found that the mRNA expression of IL-1β, IL-6 and TNFα in local plantar tissues was significantly increased after the plantar incision.[66] Peripheral inflammatory factors could cause the increase of inflammatory factors in the blood, some of which could spread to the spinal cord through the blood-brain barrier, and then caused the activation of glial cells, thus leading to spinal cord inflammation.[67]

COX-2/PG pathway is involved in the transmission of noxious stimuli at the spinal cord and plays an important role in central hyperalgesia.[65,68] Studies have found that the expression of COX-2 in the spinal cord was increased rapidly in rats with plantar incision,[65] and non-steroidal anti-inflammatory drugs could relieve hyperalgesia.[69] Flurbiprofen is widely used for postoperative analgesia. It is composed of lipid microspheres as a targeted drug delivery carrier, which can selectively accumulate in the surgical wound site.[33,70] Some animal studies have found that the administration of flurbiprofen could reduce the plantar inflammatory factors IL-6 and TNF-α in LPS-induced inflammatory pain models.[71]

It has been found that arachidonic acid, the substrate of COX1/2, can be released by the hydrolysis of endocannabinoids by fatty acid amide hydrolase and monoacylglycerol lipase.[72] CB1 receptor is the major receptors of the endocannabinoid system,[73,74] and may be a novel target to alleviate inflammation, peripheral neuropathy, and pain.[75,76] Flurbiprofen can lead to increased levels of 2-AG and AEA by inhibiting COX-2-mediated oxidation of endocannabinoids,[31,32] which are known to alleviate hyperalgesia.[77] In addition, flurbiprofen can contribute to the

activation of CB1 and CB2 receptors.[71] We found that flurbiprofen alone could increase PWL and PWMT and decrease CPS, and the mechanical and thermal pain thresholds returned to baseline at 3d and 5d after operation, respectively, which could also inhibit the activation of microglia and reduce the levels of IL-1β, IL-6 and TNF-α. This indicates that flurbiprofen axetil has a powerful analgesic effect.

Multimodal analgesia is the combination of different analgesic regimens or techniques to achieve an effective analgesic effect and reduce the consumption of opioids and their related adverse reactions.[29,78] Nerve block based analgesic program with few opioids is recommended in clinical practice. Our study also found that the combined application of nerve block and flurbiprofen not only increased PWL and PWMT and decreased CPS, but also relieved spinal cord inflammation in rats with plantar incision, and the effect was better than that of the two alone. It indicated nerve block and non-steroidal drugs had a synergistic effect. They were better than single analgesia.

In clinical practice, some studies have found that compared with general anesthesia, brachial plexus block has no significant difference in perioperative pain control and analgesic drug consumption in patients with internal fixation of distal radius fractures, and the proportion of patients who seek medical treatment again due to pain after discharge is significantly higher. Therefore, researchers suggest that patients take analgesics actively before the end of regional anesthesia to control pain.[16,79] The effect of nerve block on postoperative pain relief is limited, and the role of nerve block should be viewed rationally. In addition, some studies have found that compared with the use of nerve block alone, the combined use of nerve block and non-steroidal anti-inflammatory drugs can reduce postoperative complications, reduce VAS scores, and improve patient satisfaction.[80] Therefore, the effect of multimodal analgesia is better and more worthy of promotion. Consistent with the above clinical phenomena, this study found that the combined application of flurbiprofen and sciatic nerve block was superior to the single method, which provided a theoretical basis for the implementation of the concept of multimodal analgesia.

This study has the following limitations. First, this study did not detect the changes of inflammatory factors in the incision site and serum. Numerous studies have found that various noxious stimuli could cause the activation of spinal microglia, which in turn caused the release of many inflammatory factors and induced hyperalgesia. This study found that the earliest activation time of microglia and astrocytes was 24 hours, and the inflammatory factors were significantly increased at 6 hours, so the source of inflammatory factors needs to be further studied. Second, we did not observe the effects of adjuvant or continuous nerve block on postoperative pain. Continuous peripheral nerve block has risks of catheter obstruction, displacement, and accidental prolapse. It is difficult to control the position of the catheter in animal studies. Future studies can use adjuvant or multiple nerve blocks to explore the effect of long-term nerve block on postoperative pain.

Conclusion

In summary, to simulate a clinical scenario, the effect of preoperative sciatic nerve block on postoperative pain and spinal cord inflammation was observed in rats with plantar incision in this study. This study found that single sciatic nerve block could not improve postoperative pain or inhibit the activation of glial cells in the spinal cord, but could reduce the expression of inflammatory factors in the spinal cord. Nerve block combined with flurbiprofen could not only inhibit spinal cord inflammation but also improve postoperative pain, indicating that multimodal analgesia had a better effect, which provided a reference for the rational application of nerve block in clinical practice.

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Disclosure

The authors report no conflicts of interest in this work.

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