Coadministration of Curcumin and Hydromorphone Hydrochloride Alleviates Postoperative Pain in Rats

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This study aimed to explore the effect of curcumin and hydromorphone hydrochloride (HH) cotreatment on postoperative pain in rats. An incision + formaldehyde-induced pain rat model was established. Rats were treated with vehicle, curcumin, HH, or curcumin + HH. Paw mechanical withdrawal threshold and thermal withdrawal latency were measured at 1 d before surgery as well as 1, 2 h, 1, 3, and 7 d after surgery to assess pain sensitivity. The L4-6 region of the spinal cord was collected from each rat at 2 h, 1, 3, and 7 d after surgery. Western blot analysis and immunohistochemical staining were carried out to detect the protein expression of pain-related genes. Quantitative real-time PCR and enzyme-linked immunosorbent assay were conducted to measure the expression and production of proinflammatory mediators. Compared with other groups, Curcumin + HH significantly reduced pain sensitivity in the model rats. Mechanistically, curcumin + HH suppressed protein expression of stromal cell-derived factor-1 (SDF-1), CXC chemokine receptor 4 (CXCR4), p-Akt, and c-fos while enhancing protein expression of nerve growth factor (NGF) in the dorsal root ganglia (DRG) of model rats. Curcumin + HH inhibited the expression and production of interleukin 1β (IL-1β), cyclooxygenase-2 (COX-2), tumor necrosis factor α (TNF-α), and p65 nuclear factor kappa B (NF-κB) in the DRG. Coadministration of curcumin and HH alleviates incision + formaldehyde-induced pain in rats, possibly by suppressing the SDF-1/CXCR4 pathway and the production of proinflammatory mediators. Our results provide curcumin and HH cotreatment as a promising therapeutic strategy in the management of postoperative pain.

Key words curcumin; hydromorphone hydrochloride; postoperative pain; stromal cell-derived factor-1; CXC chemokine receptor 4; proinflammatory mediator

INTRODUCTION

Postoperative pain refers to acute pain that occurs immediately after surgery and is mainly caused by surgical incision, visceral injury, and drainage. Postoperative pain typically peaks at 24–48 h after surgery and lasts less than 7 d. However, chronic pain syndrome may develop if postoperative pain is not well controlled. Postoperative pain management includes drug therapy, regional nerve block, local anesthesia, and multimodal anesthesia, among which drug therapy is the most used method. However, side effects such as addiction, respiratory depression, nausea, and vomiting have limited the use of existing analgesic drugs. Considering the physical and mental distress in patients as well as the financial and medical burden on families and the society caused by pain, it is urgently to develop effective, low-toxic analgesic agents for pain management.

Peripheral nerve injuries arise from various types of trauma, such as laceration, compression, and drug injection injury. Peripheral nerve injury can block the sensory and motor signals between the brain and the spinal cord, leading to sensory loss and muscle weakness. Accumulating evidence has shown that traditional Chinese medicine can provide protection against neurological diseases and promote neuronal, axonal, and myelin regeneration. It has been reported that Curcuma longa protects the dorsal root ganglia (DRG) and sciatic nerve of rats from crush injury. Curcumin is the main ingredient of curcuma longa, possessing multiple pharmacological activities, such as antiinflammatory, antioxidant, antiviral, and antitumor activities, with low toxicity and minimal adverse effects. Curcumin has shown efficacy in alleviating neuropathic pain in animal models and patients. For example, curcumin treatment reduces pain sensitivity and alleviates hind paw swelling after hind paw incision in mice. Curcumin-containing mucoadhesive film ameliorates periodontal postoperative pain and swelling in patients. Moreover, curcumin relieves chronic compressive traumatic neuropathic pain by inhibiting nuclear factor kappa B (NF-κB) p65-induced CX3C receptor 1 expression in the spinal cord and DRG of rats. These findings suggest that curcumin can protect the peripheral nerve from injury, serving as a promising therapeutic drug to relieve postoperative pain. However, the underlying mechanism remains unclear.

The opioid drug hydromorphone hydrochloride (HH) is a semisynthetic derivative of morphine and is 8–10 times more potent than morphine. Due to the high analgesic potency and rapid onset of action, HH is commonly used to relieve moderate to severe pain. However, continuous pain may require higher doses of HH, leading to toxicity produced by overdose and accumulation. Recently, other drugs have been used in combination with HH to reduce the dose of HH while increasing its analgesic strength. For example, HH combined with ropivacaine has been used for labor analgesia. HH combined with flurbiprofen axetil has been used for patient-controlled intravenous analgesia in endoscopic sinus surgery. However, the effect of curcumin combined with HH on postoperative pain remains unknown.

Nerve information triggered by harmful stimuli, such as surgical wounds, travels through the peripheral nerves, the spinal cord, and the brainstem before reaching the cerebral
closely associated with posttraumatic nerve injury. Based on COX-2, tumor necrosis factor β receptor 4 (CXCR4) signaling pathway and proinflammatory mediators, including interleukin 1β (IL-1β), cyclooxygenase-2 (COX-2), tumor necrosis factor α (TNF-α), and NF-κB, are closely associated with posttraumatic nerve injury. Based on these findings, we hypothesized that curcumin might relieve postoperative pain by targeting SDF-1/CXCR4 signaling and proinflammatory mediators.

In this study, we established an inflammatory pain model in rats to explore the therapeutic effect and the underlying mechanism of curcumin on postoperative pain when used alone or in combination with HH. Our results suggest that curcumin and HH cotreatment alleviates postoperative pain, involving downregulations of SDF-1/CXCR4 signaling and proinflammatory mediator expression.

MATERIALS AND METHODS

Animals This study was approved by the Ethical Committee of The Second Hospital of Lanzhou University (ID2021-017; Gansu, China). All procedures were conducted according to the Guide for the Care and Use of Laboratory Animals implemented by the U.S. National Institutes of Health.

Clean-grade Sprague-Dawley male rats (8-week-old) weighing 200–260 g were purchased from the Laboratory Animal Centre of Lanzhou University (Gansu, China) and maintained at 20–26 °C, 40–70% humidity in a 12 h-light/12 h-dark cycle with free access to food and water. Rats were randomly divided into five groups (n = 15/group) as follows: sham, model, HH, curcumin, and curcumin + HH. All rats except those from the sham group received an incision on the sole of the right posterior paw, followed by subcutaneous injection with 2.5% formaldehyde at the incision, to establish a formaldehyde-induced inflammatory pain model. Rats in the HH group were administered 5 mg/kg HH (TD2012-0010; Yichang Humanwell Pharmaceutical, China) via intraperitoneal injection at 0.5 h before surgery. Rats in the curcumin group were administered curcumin (80% purity; C7727; Sigma-Aldrich, St. Louis, MO, U.S.A.) dissolved in dimethyl sulfoxide (DMSO) (20 mg/mL) at 100 mg/kg/d via intraperitoneal injection one day before surgery and once daily after surgery for one week. Rats in the HH group were administered both HH and curcumin + HH. 

Measurement of Paw Mechanical Withdrawal Threshold (PMWT) and Thermal Withdrawal Latency (TWL) The PMWT and TWL were measured at 1 d before surgery as well as 1 h, 2 h, 1 d, 3 d, and 7 d after surgery as previously described. Briefly, for PMWT measurement, each rat was placed in a cage with a wire mesh at the bottom. Von Frey fibers were used to vertically stimulate the area near the incision. The stimulation was stopped when the fiber was slightly bent for 4–6 s or the rat began to exhibit behavioural reactions. Each rat underwent two PMWT measurements with an interval of at least 15 s. For TWL measurement, each rat was placed on a glass plate. After the rat adapted to the environment for 30 min, the incision on the rat’s hind paw was stimulated with the irradiation light. When the rat started to withdraw the paw or the irradiation time reached 20 s, the irradiation was stopped, and the duration was recorded as the thermal pain threshold. Each rat was measured three times at a 5-min interval, and the mean value was calculated.

Enzyme-Linked Immunosorbent Assay (ELISA) ELISA was conducted to measure the levels of IL-1β, COX-2, TNF-α, and p65NF-kB in rat spinal cord tissue using corresponding ELISA kits following the manufacturer’s instructions. The IL-1β, COX-2, and TNF-α ELISA kits were purchased from Solaibao (Beijing, China). The p65NF-kB ELISA kit was from Enzyme-linked (Shanghai, China).

Western Blot Analysis The rat DRG tissue samples were ground in liquid nitrogen and lysed in radio immunoprecipitation assay (RIPA) buffer containing 1 mmol/L phenylmethylsulfonyl fluoride (Beyotime, Jiangsu, China). After centrifuging at 4 °C and 12000 r/min for 15 min, the supernatant was collected. Protein concentration was measured using the bicinchoninic acid (BCA) assay (Beyotime). A total of 20 μg proteins were separated on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred to a polyvinylidene fluoride membrane (Merck Millipore, U.S.A.). After blocking with 5% skim milk at room temperature for 1 h, the membrane was incubated with pri-
Sprague-Dawley male rats were randomly divided into sham, model, HH, curcumin, and curcumin + HH groups (n = 15/group). All rats except those from the sham group were administered formaldehyde via subcutaneous injection to induce pain, followed by treatment with vehicle, HH, curcumin, or curcumin + HH. Paw mechanical withdrawal threshold (A) and thermal withdrawal latency (B) were measured at 1 d before surgery as well as 1, 2h, 1, 3, and 7d after surgery. Data are expressed as the mean ± standard deviation (S.D.). *p < 0.05 vs. sham group, n = 15. **p < 0.01 vs. sham group; #p < 0.01 vs. model group, n = 15. (Color figure can be accessed in the online version.)

Immunohistochemical (IHC) Staining Rats were sacrificed at 2h, 1, 3, or 7d after surgery. The DRG was obtained via subcutaneous injection to induce pain, followed by treatment with vehicle, HH, curcumin, or curcumin + HH. Paw mechanical withdrawal threshold (A) and thermal withdrawal latency (B) were measured at 1 d before surgery as well as 1, 2h, 1, 3, and 7d after surgery. Data are expressed as the mean ± standard deviation (S.D.). *p < 0.05 vs. sham group, n = 15. **p < 0.01 vs. sham group; #p < 0.01 vs. model group, n = 15. (Color figure can be accessed in the online version.)
PCR showed that compared with those from sham rats, the DRG samples from vehicle-treated model rats had significantly increased mRNA levels of IL-1β, COX-2, TNF-α, and p65NF-κB through 2h to 7d after operation in a time-dependent manner (all p < 0.05; Figs. 4A–D). ELISA revealed that vehicle-treated model rats had significantly elevated serum levels of IL-1β, COX-2, TNF-α, and p65NF-κB compared with sham rats (all p < 0.05; Figs. 4E–H). Although HH or curcumin alone to
some extent reduced operation-induced expression and production of these proinflammatory mediators, treatment with curcumin + HH further diminished the expression and production of these mediators (all p < 0.05). These data suggest that coadministration of curcumin and HH suppresses proinflammatory mediator expression and production.

**DISCUSSION**

In this study, we established an incision + formaldehyde-induced pain rat model. By measuring MWT and TWL, we found that the model rats rapidly developed pain at 1 h after operation compared with the control rats, suggesting that the pain model was successfully established. We demonstrated that cotreatment with curcumin and HH significantly reduced pain sensitivity in rats compared with treatment with curcumin or HH alone, suggesting that curcumin and HH exhibit additive effects on relieving postoperative pain. Considering the critical roles of the SDF-1/CXCR4 pathway and proinflammatory cytokines in posttraumatic nerve injury, we found that the suppression of the SDF-1/CXCR4 pathway and inhibition of proinflammatory mediator production are involved in the therapeutic effect of curcumin + HH on postoperative pain.

SDF-1 belongs to the CXC family, playing multiple biological functions through activating CXCR4. SDF-1 and CXCR4 are expressed in various types of cells in the central and peripheral nervous system. The alteration in SDF-1/CXCR4 expression is involved in the pathogenesis of neurological disorders, serving as a therapeutic target for pharmacological intervention. Recent studies have implicated SDF-1/CXCR4 signaling in pain development. For example, in the DRG of rats with unilateral sciatic nerve injury-induced pain, Dubový et al. have observed SDF-1/CXCR4 upregulation that lasts for at least two weeks. Similarly, Knerlich-Lukoschus et al. have demonstrated that in a rat spinal cord injury-induced posttraumatic pain model, SDF1/CXCR4 expression levels are continuously increased at the spinal cord level from 2 to 42 d after the injury. Moreover, Reaux-Le et al. have reported that CXC4-neutralizing antibody can prevent intrathecal SDF-1 injection-induced mechanical allodynia. These findings suggest that SDF1/CXCR4 signaling contributes to the chronic and persistent pain and that targeting SDF1/CXCR4 signaling can prevent pain development.

Proinflammatory cytokines contribute to pain development. On the other hand, continuous pain stimulates inflammatory response, promoting the expression and secretion of proinflammatory cytokines. IL-1β and TNF-α are important proinflammatory cytokines that have been found to be increased in the DRG and the spinal cord in response to incision- or chronic compression-induced peripheral nerve injury. In a rat model of postoperative pain, the concentrations of IL-1β and TNF-α at the site of injury are increased within 24 to 48 h of surgery and remain elevated for 4 to 8 d after surgery. NF-κB is a key regulator of inflammation and pain. Upon activation by proinflammatory cytokines, growth factors, or other stimuli, the p65/p50 heterodimer translocates to the nucleus and initiates transcription of inflammatory genes, such as COX-2, TNF-α, and IL-1β.
as IL-1β, TNFα, and COX-2, that subsequently influence inflammation and pain.97) These multiple molecules and mechanisms become novel therapeutic targets for analgesic drug development.98) To investigate the effect of curcumin + HH on proinflammatory mediator production, we measured the expression of IL-1β, COX-2, TNF-α, and p65NF-κB in the DRG of model rats. The results showed that treatment with curcumin + HH significantly reduced the mRNA and protein levels of these proinflammatory mediators compared with other groups, suggesting that cotreatment with curcumin and HH could alleviate postoperative pain by suppressing inflammatory response during pain development.

Neurotrophins NGF promotes the survival of sensory and sympathetic neurons.99) We found that NGF protein level in the curcumin + HH group was remarkably higher than that in other groups, suggesting that curcumin + HH may facilitate regeneration and repair of neurons and thus relieving postoperative pain. The proto-oncogene c-fos is a marker of nociceptive neuronal activity.40) Our results showed that c-fos expression in the curcumin + HH group was significantly lower than that in other groups, suggesting that nociceptive neuronal activity is decreased in response to curcumin and HH cotreatment. However, the underlying mechanism needs further investigation.

In conclusion, we demonstrate that coadministration of curcumin and HH could alleviate incision + formaldehyde-induced pain in rats. Curcumin combined with HH could suppress the expression of SDF-1, CXCR4, p-Akt, c-fos, and proinflammatory mediators while enhancing the expression of NGF, suggesting that curcumin combined with HH alleviates postoperative pain possibly by inhibiting inflammation and promoting neuron repair. Our results provide curcumin combined with HH as a promising therapeutic strategy in the management of postoperative pain.

Author Contributions Yihan Wang designed the study. Yihan Wang, Yang Liu, Yingbin Wang performed the experiments and drafted the manuscript. Yihan Wang, Jieting Liu, Min Wang, analyzed and interpreted the data. All authors read and approved the final manuscript.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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