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Original Article

Pain control after intrathecal MCP-1 neutralizing antibody injection into rat models of tumor cell injection-induced bone pain and morphine tolerance and possible mechanism

Lei Liu, Fen-Fen Bai, Lei Feng, Xian-Wen Liu, Ping Zhang, Chun-Guang Ren, Zong-Wang Zhang, Yong Zhao

Department of Anesthesiology, Liaocheng People's Hospital, Liaocheng 252000, Shandong, China

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Abstract: Background: Cancer-induced bone pain commonly occurs in patients with metastasized breast, prostate, or lung cancer. Opioids are the first-line therapy, but opioid tolerance and addiction limit their usage and increase the dosage. The aim of this study was to assess the efficacy of anti-monocyte protein-1 (MCP-1) neutralizing antibodies in the control of cancer pain, as well as the mechanism, in rat models of breast cancer-induced bone pain and morphine tolerance. Methods: The rat model of Walker 256 tumor cell injection-induced bone pain utilized 72 female Sprague-Dawley rats, which were randomly divided into six groups (n=12); while the rat model of morphine tolerance included 48 rats. Pain in the rats was assessed by paw withdrawal threshold (PWT) and thermal withdrawal latency (TWL) tests, and rat spinal cord tissues were also analyzed immunohistochemically with the OX-42 antibody against CH11b and enzyme-linked immunosorbent assay (ELISA) detection of interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α). Results: There were significant increases in the PWT and TWL duration after intrathecal administration of morphine on day 12, but these values decreased between days 15 and 18, indicating that morphine tolerance occurred in the animals. In contrast, treatment with the anti-MCP-1 neutralizing antibody markedly reduced this morphine tolerance. At the protein level, there was low expression of CD11b, TNF-α, IL-1, and IL-6 in the spinal cords of morphine-resistant rats after intrathecal injection of the MCP-1 neutralizing antibody, compared to the control group. Conclusion: These findings suggest that MCP-1 may activate bone microglial cells to trigger inflammation and pain as well as morphine tolerance in cancer-induced bone pain.

Keywords: Opioids, tolerance, MCP-1, bone cancer, inflammation, morphine tolerance

Introduction

Mortality and morbidity due to cancer are continuously increasing throughout the world, including China, for both men and women [1, 2]. Encouragingly, significant advancements in the early detection of cancer and new therapies have led to better survival of cancer patients [3]. However, continuously improved survival rates have led to an increasing number of patients living with cancer-associated pain [4, 5]; which significantly impacts the quality of life of these patients [4]. To date, opioids remain the first-line treatment for cancer pain [6, 7], although such medications have at least two limitations, i.e., the development of drug tolerance and addiction [6, 7], which limit their usage and increase the dosage to manage cancer-induced pain in patients. Thus, the discovery of better agents and therapies for cancer pain is urgently needed. Currently, the animal model of morphine tolerance forms the basis for research on the biological mechanisms of drug tolerance and novel agents. Nevertheless, this animal model does not closely mimic the clinical scenario [8]. Cancer pain is one of the most complex forms of chronic pain, as it involves multiple mechanisms including both inflammatory and neuropathic pain [5]. Pain is the most common symptom in patients with bone cancer [9]; thus, a rat model of bone cancer pain could closely mimic the clinical scenario, making it a better model for cancer pain research [10]. Opioid tolerance has been well documented with regard to morphine, which is the most commonly prescribed drug in clinical practice for cancer-associated pain [11].

Previous studies have suggested that the molecular mechanisms underlying neuropathic pain may be analogous to that of morphine tolerance, both of which may be related to an increase in hyperalgesia [12]. Morphine tolerance may result from microglial cell activation [3, 13, 14]. In addition, inflammatory cytokines play a central role in regulating chronic pain [15, 16]. For example, interleukin (IL)-1 and IL-6, well-known mediators in the neuroendocrine immune system, are sensitive indicators of the acute inflammatory reaction; and nociceptive stimuli may elicit heightened levels of IL-1 and IL-6, which are associated with increased pain [17]. Moreover, tumor necrosis factor-alpha (TNF-α) causes hyperalgesia by inducing cyclooxygenase-2-catalyzed prostaglandin E synthesis and also by stimulating the release of bradykinin, substance P, and catecholamines, as well as the activation of TNF-α, IL-1, and IL-6 receptors on neurons and glial cells, resulting in heightened nerve excitability, which increases central sensitization and hyperalgesia [18]. Furthermore, monocyte chemoattractant protein-1 (MCP-1) is a chemokine that binds to the C-C chemokine receptor type 2 (CCR2) [19]. Currently, there are multiple phase I/II clinical trials that have assessed selective MCP-1 antagonists for the treatment of inflammatory diseases, atherosclerosis, and cancer [20, 21]. The increased expression of MCP-1 during inflammation along with the reduced inflammatory hyperalgesia observed after treatment with an anti-MCP-1 antibody suggest that this chemokine likely plays a role in inflammatory hypernociception. In addition, upregulated MCP-1 expression has been demonstrated in macrophages from paws of carrageenan- or complete Freund's adjuvant-treated mice, and the inflammatory hyperalgesia was prevented using an anti-MCP-1 serum [22].

Therefore, in this study, we first established rat models of tumor cell injection-induced bone pain and morphine tolerance, and then assessed the pain levels using the paw withdrawal threshold (PWT) and thermal withdrawal latency (TWL) tests after intrathecal injection of an anti-MCP-1 antibody. We then analyzed the expression of CD11b a microglia marker in the brain using the OX-42 antibody [3, 23], as well as TNF-α, IL-1, and IL-6 in the spinal cords of the rats.

Materials and methods

Animals

This study was approved by the Animal Protection Committee of Shandong University (approval number: SYXK (Lu) 2013 0009), and the animal experiments were conducted in accordance with the regulations of the International Association of Pain Research [24]. A total of 120 adult female Sprague Dawley rats with a body weight of 150-180 g were purchased from the Laboratory Animal Center, Chinese Academy of Medical Science (Beijing, China). The animals were housed at a room temperature between 22°C and 24°C with 40-60% relative humidity under a 12/12-h light dark cycle with free access to food and water *ad libitum*.

Animal model of tumor cell injection-induced bone pain

A breast cancer cell-induced bone pain model was established by injection of Walker 256 cells into the marrow cavity of the rat tibia, according to previous studies [25, 26]. Briefly, tumor cells originally obtained from the Chinese Academy of Medical Sciences (Beijing, China) were grown and harvested from the ascites of rats to cultivate 20 million cells/mL in Hank's balanced salt solution (Sigma Aldrich, St. Louis, MO, USA). Next, the rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (Sigma Aldrich) at a dose of 40 mg/kg and injected with 10 μL of Hank's balanced salt solution containing 1×10^5 Walker 256 cells into the marrow cavity of the rat tibia. A total of 72 rats were randomly divided into six groups (n=12): bone cancer pain (B), morphine-resistant bone cancer pain (BM), morphine-resistant bone cancer pain receiving intrathecal injection of the MCP-1 neutralizing antibody (BM-Ab), morphine-resistant bone cancer pain receiving intrathecal injection of IgG (BM-IgG), bone cancer pain receiving intrathecal injection of the MCP-1 neutralizing antibody (B-Ab), and bone cancer pain receiving intrathecal injection of IgG (B-IgG). Specifically, the animals in group B were injected with tumor cells into the medullary cavity of the tibia; and on day 9, 4 μL of normal saline was intrathecally injected twice a day for nine days. For the BM group, after Walker 256 cell injection, the rats were also

administered 20 μg/kg morphine intrathecally twice a day from day 9 to day 18. The rats in the BM-Ab group received Walker 256 cell injection and an intrathecal injection of 20 μg/kg morphine twice a day from day 9 to day 18 as well as a daily intrathecal injection of 10 μg of the MCP-1 neutralizing antibody starting on day 15, for three days. Meanwhile, the rats in the BM-IgG group underwent the same procedures as the BM-Ab group but with nonspecific IgG injection to replace the anti-MCP-1 antibody. Moreover, the animals in the B-Ab group underwent the same procedure as the BM-Ab group but without morphine injection, and the B-IgG group was used as a negative control, i.e., the animals with tumor cell injection-induced bone cancer pain were only treated with 4 μL of normal saline twice a day to replace the morphine and with nonspecific IgG to replace the anti-MCP-1 antibody.

Rat model of morphine-tolerant cancer cell-induced bone pain

We first established the breast cancer cell-induced bone pain model by injection of 1×10^5 Walker 256 cells into the tibia, according to previous studies [25, 26]. The morphine tolerance in the animals was then induced by injection of 20 μg/kg morphine (Sigma Aldrich) twice a day from day 9 to day 18. On day 18, all animals were sacrificed, and the spinal cord tissues were resected and stored at -80°C until use for morphological analysis and immunohistochemistry.

The paw withdrawal threshold (PWT) test

Both animal models induced pain. Mechanical allodynia was measured by using Von Frey filaments (Stoelting Co., Wood Dale, IL, USA), according to previous studies [25, 26]. In brief, the pain was measured before tumor cell injection and 3, 6, 9, 12, 15, and 18 days after cell injection. The animals were placed in individual plastic boxes (20 cm × 25 cm × 15 cm) containing a metal mesh floor and allowed to acclimate for 30 min. The pain level was assessed by stimulating the plantar surface with the Von Frey filaments for 6-8 s, and animals with a brisk withdrawal or paw flinching were considered as having a positive response. The threshold measurement of pain was assessed by using the up-down paradigm, a powerful tool for analyzing the pain state, according to a previous study [27].

The thermal withdrawal latency (TWL) test

Both animal models were also assessed for heat hypersensitivity using a plantar tester (type 7370; UgoBasile, Varese, Italy), as described previously [28]. The animals were assessed before tumor cell injection and 3, 6, 9, 12, 15, and 18 days after cell injection by placing the animals on a glass floor and then positioning a radiant heat source beneath the glass floor. The test was defined as the time (in seconds) between the delivery of the thermal stimulus and withdrawal of the hind paw. Three measurements were recorded for each hind paw in each test session, and the test was alternated at 5-min intervals between consecutive tests. The mean latency of the three measurements per side was summarized.

Immunohistochemistry

The OX-42 antibody (Cat. #SC-7898) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and the SP kit (Cat. #60582471) was obtained from Beijing Zhongshan Biotechnology Co., Ltd. The spinal cord tissues were removed from the -80°C freezer and warmed up to room temperature in a 20% sucrose solution overnight. After that, 40-μm-thick tissue sections were prepared using a cryotome and washed three times for 5 min each with phosphate-buffered saline (PBS). Next, the tissue sections were incubated with 3% H_2O_2 in PBS for 5 min, washed three times with PBS for 5 min each, and then blocked in 10% normal goat serum in PBS at room temperature for 30 min. The sections were then incubated with the primary antibody at a dilution of 1:400 at 4°C overnight, followed by washing three times with PBS for 5 min each. The biotin-labeled secondary antibody, diluted in 1% bovine serum albumin-PBS was added onto the sections, and the sections were incubated at 37°C for 30 min. Subsequently, the sections were incubated with horseradish peroxidase-labeled streptavidin diluted in PBS at 37°C for 30 min before the colorimetric reaction using 3,3'-diaminobenzidine solution. The immunostained tissue sections were then dehydrated in a series of 75-100% ethanol solutions for 5 min each and clarified in xylene for 10 min, before being sealed with a coverslip using a neutral gum. The positive OX-42 antibody staining was reviewed and scored under an inverted microscope (Olympus-CKX41), and five fields of each section were photographed with a mounted

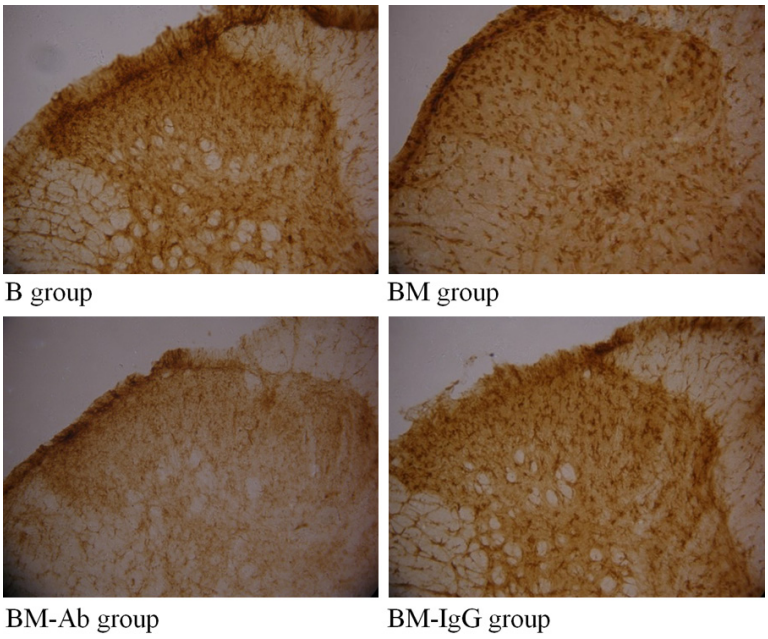

Figure 1. Spinal cord expression of CD11b in each group of rats. Spinal cord tissues were immunostained with the OX-42 antibody to visualize the microglia in each group of rats (400 × original magnification).

Olympus camera (C7070wz, Olympus, Shinjuku, Tokyo, Japan). Image-Pro Plus 4.5 software (Media Cybernetics, Rockville, MD, USA) was used to analyze these images, and the number of positive cells was recorded. The integrated optical density (IOD) in each field was obtained separately, and the average values of the positive cell number (NUM) and IOD were calculated.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA kits to detect TNF-α (cat. #bsk001-62), IL-1 (cat. #JK-a-0006), and IL-6 (cat. #QN-PS1726) were purchased from Shanghai Xin Yu Biological Technology Co., Shanghai Crystal Anti-Biological Engineering Co., and Shanghai Qiao Yu Biotechnology Co., respectively, and performed according to the manufacturers' protocols. Specifically, we first stored these antibody-coated ELISA plates at 4°C overnight. On the next day, we washed them three times with PBS-Tween 20 (PBS-T) and incubated them with 10% calf serum at 4°C overnight before the addition and incubation with the primary antibody at 37°C for 2 h. After washing three times with PBS-T, the plates were then incubated with the secondary antibody. The reaction was visualized with a colorimetric reaction, which was measured by using a spectrophotometer (Metash, Shanghai, China) at 0.2 nm.

Statistical analysis

All data were expressed and summarized as the mean ± standard deviation and statistically analyzed using the SPSS 16.0 statistical software package (SPSS, Chicago, IL, USA). The data were compared and analyzed using one-way analysis of variance, and a *p*-value less than 0.05 was considered statistically significant.

Results

Association of MCP-1 with hyperalgesia

In this study, we first established rat models of tumor cell injection-induced bone pain and morphine-tolerant cancer cell-induced bone pain, and then assessed pain levels using the PWT and TWL tests. This study intended to investigate the involvement of MCP-1 in hyperalgesia in these rat models by intrathecal injection of an anti-MCP-1 antibody on day 15 after Walker 256 cell inoculation. The PWT and TWL test data showed a significant increase in durations on day 12 after intrathecal administration of morphine for 3 days; however, such durations were decreased between days 15 and 18 after daily intrathecal administration of morphine (i.e., day 18 after cell injection; **Figure 2**), indicating morphine tolerance in the animals. Our data further showed that the anti-MCP-1 neutralizing antibody could markedly reduce the morphine tolerance in these animals as shown by the reduction of tolerance to heat and filaments compared with those of control IgG; **Figure 2**).

Differential CD11b (OX-42) expression in the spinal cords of morphine-resistant rats after intrathecal injection of the MCP-1 neutralizing antibody

We then assessed the expression of CD11b, a microglia marker, in the spinal cords of the BM-Ab group using the OX-42 antibody [3, 23]. The positive cell number (17.33±1.87) and the IOD (350.91±30.97) were significantly lower ($P<0.05$) in the BM-Ab group compared with the corresponding values of 34.76±2.17 and

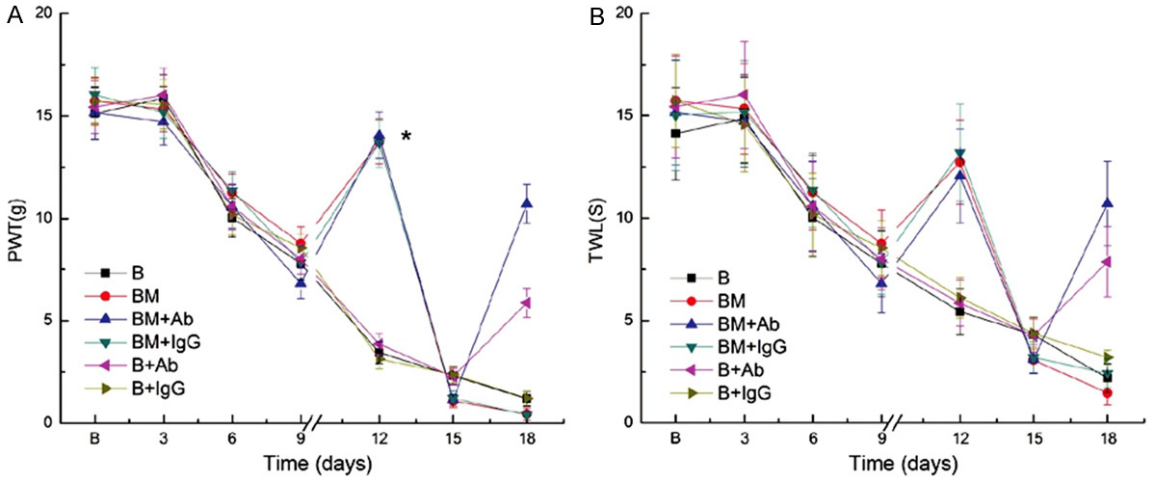

Figure 2. Effects of intrathecal injection of anti-MCP-1 neutralizing antibody on the regulation of mechanical allodynia in rats. (A) PWT and (B) TWL values were measured between days 1 and 18 after tumor cell inoculation. Morphine was intrathecally injected between days 9 and 18 in all BM groups, and a single intrathecal administration of anti-MCP-1 antibody (BM+Ab and B+Ab groups) or control IgG (10 μg; BM+IgG and B+IgG groups) was performed on day 15 after inoculation of Walker 256 cells. The values are presented as the mean ± standard error (n=8). *P<0.05 vs. the BM and BM+IgG groups. PWT, the paw withdrawal threshold test; TWL, the thermal withdrawal latency test; B, bone cancer pain group; BM, morphine tolerance and bone cancer pain group.

Table 1. CH11b (OX-42) expression in the rat spinal cords (mean ± SD; n=6)

Group	NUM	*P* value	IOD	*P* value
B	22.47±1.67		451.77±26.03	
BM	34.76±2.17	0.04	653.12±43.58	0.03
BM-Ab	17.33±1.87*	0.02	350.91±30.97*	0.03
BM-IgG	33.42±2.58	0.32	638.05±38.91	0.41
B-Ab	19.14±1.51	0.01	372.56±28.19	0.02
B-IgG	23.12±1.98	0.34	447.97±32.67	0.14

*Compared with that of the BM group. B, bone cancer pain; BM, morphine-resistant bone cancer pain; BM-Ab, morphine-resistant bone cancer pain receiving intrathecal injection of the MCP-1 neutralizing antibody; BM-IgG, morphine-resistant bone cancer pain receiving intrathecal injection of IgG; B-Ab, bone cancer pain receiving intrathecal injection of the MCP-1 neutralizing antibody; B-IgG, bone cancer pain receiving intrathecal injection of IgG; NUM, the positive cell number; IOD, the integrated optical density.

653.12±43.58 in the BM group (**Figure 1** and **Table 1**). However, there was no statistically significant difference in CD11b expression between the B and B-Ab groups, the B and B-IgG groups, or the BM and BM-IgG groups (**Figure 1** and **Table 1**).

Differential TNF-α, IL-1, and IL-6 expression in the spinal cords of morphine-resistant rats after intrathecal injection of the MCP-1 neutralizing antibody

Next, we detected the levels of TNF-α, IL-1, and IL-6 in the spinal cords of the animals and found that their levels were all significantly lower in the BM-Ab group (2.03±0.67, 2.03±0.67, 2.21±0.51, respectively; *P*< 0.05) compared with those in the BM group (7.65±1.76, 8.08±2.03, and 7.89± 1.24, respectively; **Table 2**). However, there was no statistically significant difference in the TNF-α, IL-1, or IL-6 level between the B and B-Ab groups, the B and B-IgG groups, or the BM and BM-IgG groups (**Table 2**).

Discussion

Cancer pain is one of the most complex forms of chronic pain as it is a combination of both inflammatory and neuropathic pain [5]. Bone cancer pain is the most difficult type of pain to control and possibly the most serious form of cancer pain. During the early stages of bone cancer, tumor cells grow in the bone marrow cavity and stimulate osteoclasts, causing an imbalance between osteolysis and osteogenesis. This result in bone destruction, which may be the mechanism by which bone cancer pain is induced [29]. During the advanced stages of bone cancer, the disease rapidly expands and the tumor volume increases, causing the periosteum to be pulled away from the bone surface. Cancer can also directly erode and damage the peripheral nerves, causing nerve damage, oppression, and

Table 2. Expression of TNF-α, IL-1, and IL-6 in the spinal cords of the different groups

Group	TNF-α	*P* value	IL-1	*P* value	IL-6	*P* value
B	4.23±1.10		4.18±1.04		4.56±0.97	
BM	7.65±1.76	0.04	8.08±2.03	0.02	7.89±1.24	0.03
BM-Ab	2.03±0.67*	0.03	2.03±0.67*	0.01	2.21±0.51*	0.02
BM-IgG	8.01±1.69	0.11	7.94±1.92	0.52	8.12±1.52	0.27
B-Ab	2.64±1.08	0.03	3.03±0.67	0.01	2.98±0.73	0.02
B-IgG	4.01±1.26	0.29	3.98±1.17	0.13	4.83±1.21	0.58

*Compared with that of the BM group. B, bone cancer pain; BM, morphine-resistant bone cancer pain; BM-Ab, morphine-resistant bone cancer pain receiving intrathecal injection of the MCP-1 neutralizing antibody; BM-IgG, morphine-resistant bone cancer pain receiving intrathecal injection of IgG; B-Ab, bone cancer pain receiving intrathecal injection of the MCP-1 neutralizing antibody; B-IgG, bone cancer pain receiving intrathecal injection of IgG; NUM, the positive cell number; IOD, the integrated optical density.

ischemia. Additionally, cancer cells can secrete proteases that trigger proteolysis of fibers and sympathetic fibers, leading to neuropathic pain [30]. In the primary afferent fibers of the dorsal horn of the spinal cord in rats with bone cancer pain, there was a significant increase in the number of astrocytes, accompanied by an upregulation of c-fos expression and dynorphin in deep neurons of the spinal dorsal horn [31]. This also shows that peripheral sensitization of sensory afferent pathways and central sensitization constitute important mechanisms of bone cancer pain [32, 33]. For example, Li *et al*. [34] have shown that an increased expression of receptor activator of nuclear factor kappa-B ligand is directly involved in osteoclast-induced pain in a rat model of bone cancer pain. In addition, osteoclasts create a slightly acidic microenvironment for bone resorption, whereas the sensory neurons can be directly activated by acid or hydrogen ions [34]. Clinically, the pain associated with osteoclasts is directly related to bone destruction, and the two major acid-sensitive ion channels expressed on nociceptors are acid-sensing ion channel 3 and transient receptor potential vanilloid/transient receptor potential cation channel subfamily V member 1/capsaicin receptor/vanilloid receptor 1 (TRPV1) [35]. TRPV1 plays an important role in mechanical allodynia and thermal hyperalgesia [36]. Thus, to investigate the potential mechanisms of morphine tolerance in cancer pain, we first established an animal model to mimic the actual clinical situation. Our data showed a significant increase in the PWT and TWL durations after intrathecal administration of morphine on day 12, but a decrease between days 15 and 18, indicating that morphine tolerance occurred in the animals. In addition, treatment with the anti-MCP-1 neutralizing antibody markedly reduced this morphine tolerance in rats. Moreover, the expression of CD11b (OX-42), TNF-α, IL-1, and IL-6 was low in the spinal cords of morphine-resistant rats after intrathecal injection of the MCP-1 neutralizing antibody vs. that of the control rats, suggesting that MCP-1 was able to activate bone microglial cells to trigger inflammation and pain as well as morphine tolerance in cancer-induced bone pain. Future studies will further assess MCP-1 antagonists in the management of patients with bone cancer pain.

Indeed, inflammatory mediators in the tumor lesions can contribute to cancer pain development, like high-mobility group protein-1 (HMGB1) and IL-1β, which are simultaneously expressed in the dorsal horn of the spinal cord, and intrathecal injection of the HMGB1 neutralizing antibody can effectively antagonize the tactile and hemorrhagic pain responses; therefore, bone tumors have increased expression of IL-1β in the spinal cord after induction of HMGB1 [37]. In turn, this modulates the signal transmission and pain response of spinal excitatory synapses, and a variety of signal transduction molecules and ion channels do play crucial roles in pain signaling. Recently, small-diameter primary afferent fibers have been shown to stimulate glutamate, substance P, and calcitonin gene-related peptide production. In addition, the NR2B-containing N-methyl-D-aspartate (NMDA) receptors in the presynaptic membrane facilitate and maintain the transduction of nociceptive signals through lease of the neurotransmitters to associate with the increased excitability of neurons. Thus, it was speculated that an increase in NR2B-containing NMDA receptor expression could play an important role in the generation and maintenance of bone cancer pain in mice [38]. Furthermore, it is well documented that the neuronal excitability, at least in part, relies on the activation threshold of sodium channels, along with the intrinsic properties of the channels, such as transport, density, and distribution. A previous

study has shown that the tetrodotoxin-resistant peripheral nerve (TTX-RNav1.8) sodium channel is exclusively expressed on peripheral sensory neurons, RNav1.8 expression in the dorsal root ganglion neurons is decreased, the TTX-R sodium current is diminished, and the maintenance of bone cancer pain may be linked to Nav1.8 sodium channels [39]. Additionally, intrathecal injections of Nav1.8 antisense oligonucleotides to suppress Nav1.8 sodium channel activity have been demonstrated to inhibit hyperalgesia and bone cancer pain, and animal behavioral tests have shown that the Nav1.8 sodium channel is likely involved in the maintenance of bone cancer pain [39]. Another previous study [40] analyzed the involvement of the chemokine Fractalkine in bone cancer pain, while additional studies have suggested that the gamma-aminobutyric acid B receptor might also play a role in mediation of tibial cancer pain by regulation of downstream p-ERK1/2 signaling [41]. In addition, lysophosphatidic acid, a phospholipid signaling molecule, has been shown to sensitize bone cancer pain through sensitization of the peripheral C nerve [42]. In bone cancer pain models, calcium/calmodulin-dependent protein kinase II and mitogen-activated protein kinase p38 were shown to be involved in the production and maintenance of bone cancer pain [43] as p38 produced arachidonic acid through phospholipase A2 activation and subsequently underwent metabolism by cyclooxygenases 1 and 2 to produce prostaglandins, in turn activating the dorsal horn of the spinal cord. Thus, intrathecal injection of p38β antisense oligonucleotide attenuated thermal hyperalgesia and mechanical hyperalgesia in rats with bone cancer pain.

It has been shown that OX-42^{+} cells and p-Akt levels were increased in the microglia and spinal dorsal horns of rats with bone cancer pain [23], but they were suppressed by intrathecally administered MCP-1 neutralizing antibody or an inhibitor of phosphatidylinositol 3-kinase [23]. Meanwhile, the mechanical allodynia subsided after treatment with the inhibitor, and activation of the spinal microglia induced by MCP-1 occurred through a pathway involving phosphatidylinositol-3-kinase and p-Akt in bone cancer pain. Moreover, Zhao *et al*. [44] have observed that intrathecally administered MCP-1-neutralizing antibody also diminished morphine tolerance by inhibition of the prolonged morphine stimulation to the spinal microglia. Thus, neuronal MCP-1-stimulated spinal microglia may be involved in the development of morphine tolerance during the treatment of bone cancer pain, whereas inhibition of MCP-1 could provide a novel therapeutic option for morphine tolerance management. In addition, Yao *et al*. [45] have reported that an antibody against nerve growth factor mitigated hyperalgesia in rats with bone cancer pain that was associated with an increased number of μ-opioid receptors. Furthermore, the cyclooxygenase-2 inhibitor DFU has been demonstrated to inhibit tumor pain by suppression of MCP-1 production in tumor tissues and the blood circulation [46].

It has been reported that hyperalgesia associated with cancerous bones is stimulated by HMGB1 phosphorylation through protein kinase C to lead to its exit from the cellular nucleus and liberation from the cytoplasm of the dorsal horn, resulting in upregulation of inflammatory mediators in the spinal cord [47]. Moreover, the TNF inhibitor etanercept decreases bone cancer pain through the Mas-related gene receptor C (MrgC)-N-methyl D-aspartate receptor subtype 2B (NR2B) signaling pathway, as evident by downregulation of inflammatory factors, like nitric oxide synthases, MrgC, and NR2B, in an established bone cancer pain model [48]. Additionally, bone cancer pain can be treated with μ-opioid agonists, such as fentanyl, and other κ-opioid receptor agonists (e.g., U50, 488) [49]. Furthermore, intrathecal administration of the endothelin A receptor antagonist BQ-123 alleviates bone cancer pain by inhibition of Akt and ERK signaling [50], while the β-fiber non-nociceptive primary sensory neurons in the dorsal root ganglion of the spinal cord likely play a role in the peripheral sensitization and cancer-elicited tactile excitability in bone cancer pain [51]. The voltage-dependent anion channel 1 also has been shown to play a role in tumor progression and bone cancer pain by upregulation of TLR4 expression [52]. Thus, it appears that the mechanisms underlying cancer-induced bone pain may be a complicated network rather than a single pathway.

MCP-1 may activate spinal microglia through the PI3K/Akt pathway [44], and upregulation of spinal MCP-1 and CCR2 levels has been implicated in mechanical allodynia linked to bone

cancer pain [53]. However, augmented spinal MCP-1 and CCR2 expression may also play a role in mechanical allodynia in rats exhibiting bone cancer pain and morphine tolerance [54]. Systemic administration of tumor cells in the earlier animal models of bone cancer pain resulted in poor animal health and the development of tumor metastases; thus, our current animal models are better [55] and closely mimic the clinical situations. Our current findings provide insight into novel strategies for tackling the problem of opioid tolerance in the treatment of cancer and related diseases.

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Disclosure of conflict of interest

None.

Address correspondence to: Yong Zhao, Department of Anesthesiology, Liaocheng People's Hospital, 67 Dongchang West Road, Liaocheng 252000, Shandong, China. Tel: +86-01186-6358503624; E-mail: zhuliulei@163.com

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