MCP-1 Regulates Fractalkine Secretion through p38MAPK Signaling Pathway in Rats with Advanced Bone Cancer Pain

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To explore the effect and mechanism of monocyte chemoattractant-1 regulating fractalkine secretion of neurons through p38mitogen-activated protein kinase signaling pathway in rats with advanced bone cancer pain. 30 adult female Sprague-Dawley rats of clean grade were selected and 5 Sprague-Dawley rats were selected as sham group. The rest rats were injected Walker 256 tumor cells to establish rat bone cancer pain model. After successful modeling, some rats were divided into 6 d group, 12 d group and 18 d group at random. The monocyte chemoattractant-1 protein in spinal cord was compared at 0 h, 1 h, 2 h, 4 h and 24 h. The rest of the model rats were randomly divided into bone cancer pain group and monocyte chemoattractant-1 group. The rats in the bone cancer pain group were not treated. The monocyte chemoattractant-1 rats were intrathecally injected with 10 μg/μl monocyte chemoattractant-1 neutralizing antibody. After 12 d, the pain threshold PWT, phopho-p38, chemokine C-X3-C-motif receptor 1 protein expression levels and fractalkine secretion were detected in sham group, bone cancer pain group and monocyte chemoattractant-1 group 12 d later. The monocyte chemoattractant-1 protein in spinal cord of 6 d group, 12 d group and 18 d group was higher than sham group. After 12 d, PWT value of bone cancer pain group was lower than sham group. The PWT value of monocyte chemoattractant-1 group was higher than bone cancer pain group. The phopho-p38, chemokine C-X3-C-motif receptor 1 and fractalkine secretion in spinal cord of bone cancer pain group were higher than sham group. The phopho-p38, chemokine C-X3-C-motif receptor 1 and fractalkine secretion in spinal cord of monocyte chemoattractant-1 group were lower than bone cancer pain group. The monocyte chemoattractant-1 is up-regulated in rats with advanced bone cancer pain, which can regulate the secretion of fractalkine by activating p38 mitogen-activated protein kinase signaling pathway, and then act on chemokine C-X3-C-motif receptor 1 and participate in the process of bone cancer pain in rats.

Key words: Monocyte chemoattractant-1, p38mitogen-activated protein kinase, fractalkine, bone cancer

Bone Cancer Pain (BCP) is a common type of pain, which has a unique mechanism of onset and progression. Due to the lack of awareness of the disease, it significantly lowers the patient's quality of life[1]. In 1986, the World Health Organization first introduced a three-step treatment method for cancer pain, but a great deal of cancer patient's pain has not yet been significantly reduced, which may be due to the fact that the analgesic effect of cancer pain control drugs is not ideal, and there are many adverse reactions, which cannot be used for a long time[2]; therefore, it is a hot topic to further explore the spine of cancer pain and find new and effective analgesic targets. Many reports have shown that immune cells in the central nervous system cannot only maintain and nourish the growth and development of neurons, but also activate glial cells to secrete many chemicals. After being mediated by neurotransmitters, the pain signal is transmitted, which eventually leads to the aggravation of pain response[3,4]. Fractalkine (FKN, CX3CL1) is a new chemokine, which can be divided into two types, membrane binding and soluble. It is mainly expressed on neurons and can participate in information transmission between neurons and microglia[5]. In recent years, the role

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of Monocyte Chemoattractant Protein-1 (MCP-1) in pain has attracted people's attention. Many studies have found that the expression of MCP-1 in neuropathic animal models is increased, which has the role of generating and maintaining neuropathic pain[6,7]. p38 signaling pathway is a Mitogen Activated Protein Kinase (MAPK) signal pathway. As one of the more thoroughly studied pathways, p38 signal pathway can be activated by stress factors, enter the nucleus from the cytoplasm, and finally participate in the process of cell proliferation and apoptosis and play an important role[8,9]. However, there are few studies on the role of MCP-1, p38MAPK signaling pathway and FKN in advanced BCP. Thus, in rats with advanced BCP, the aim of this work is to investigate the role and mechanism of MCP-1 in controlling FKN secretion of neurons *via* the p38MAPK signaling pathway. Thirty adult female Sprague-Dawley (SD) rats of clean grade were purchased from Beijing Taize JIAYE Technology Development Co., Ltd., with production license No: scxk (Jing) 2018-0006. All rats were raised in cages with 5 rats in each cage. They were free to eat and drink water. They were replaced every 12 h day and night. The temperature and humidity were set at (25°±3°), (50±5) %. After three consecutive days of behavioral testing, the rats were fed in an adaptable manner within a test box made of plexiglass for 7 d. Mouse anti MCP-1, phopho (p)-p38 and CX3CR monoclonal antibodies were from Shanghai qunji Biotechnology Co., Ltd.; Bicinchoninic Acid (BCA) protein quantitative kit was from Wuhan yunclonal Diagnostic Reagent Research Institute Co., Ltd.; MCP-1 neutralizing antibody was from R & D systems; Normal goat Immunoglobulin G (IgG) was from Luoyang Baitaike Biotechnology Co., Ltd.; mouse anti Glial Fibrillary Acidic Protein (GFAP) primary antibody was from Beijing Baiaolaibo Technology Co., Ltd.; Walker 256 cancer cell line was purchased keelton Biotechnology (Shanghai) Co., Ltd. Low speed automatic balance centrifuge was purchased from Guangzhou Beimate Instrument and Equipment Co., Ltd.; purification console was from Beijing Taize Jiaye Technology Development Co., Ltd.; inverted microscope was purchased from Nanjing Beiden Medical Co., Ltd.; Roswell Park Memorial Institute (RPMI) 1640 culture medium was purchased from Hangzhou Jinuo Biomedical Technology Co., Ltd.; rat observation box was purchased from Hua'an Medical Co., Ltd.; fluorescence microscope was purchased from Beijing Taize Jiaye Technology Development Co., Ltd., Purchased from Guangzhou Mingmei Photoelectric Technology Co., Ltd. Five SD rats were chosen at random to form the sham group. The remaining rats were injected Walker 256 tumor cells to establish rat BCP model. After successful modeling, some rats were divided into 6 d group, 12 d group and 18 d group. In sham group, the rats were decapitated at 6, 12 and 18 d after injection, and their spinal cord L4~L6 was taken from ice and the MCP-1 protein in spinal cord was detected by Western blot. The rest of the model rats were divided into BCP group and MCP-1 group at random. The rats in BCP group were not treated. MCP-1 rats were intrathecally injected with 10 μg/μl MCP-1 neutralizing antibody. After 12 d, the mechanical pain threshold PWT of sham group, BCP group and MCP-1 group at 0 h, 1 h, 2 h, 4 h and 24 h were detected. The rats in each group were decapitated and sacrificed. The L4~L6 spinal cord was taken from the ice. The p-p38 and C-X3-C Motif Chemokine Receptor 1 (CX3CR1) were detected by Western blot, and the FKN secretion was detected by immunofluorescence staining. Rat spinal cord MCP-1 and p-p38 protein expression levels were represented as (x±s) for each group. A one-way Analysis of Variance (ANOVA) was utilized for multi group comparison, and the t-test was employed to compare the two groups. $p<0.05$ was considered to be a statistically significant difference in all data analysis using Statistical Package for the Social Sciences (SPSS) 22.0. Compared with sham group, $p<0.05$ and that compared with BCP group, $^{\#}p<0.05$. The expression of MCP-1 protein in spinal cord of 6 d group, 12 d group and 18 d group was higher than sham group as shown in Table 1. After 12 d, PWT value of BCP group was reduced than sham group. The PWT value of MCP-1 group was higher than BCP group as shown in Table 2. The p-p38, CX3CR and FKN secretion in spinal cord of BCP group were higher than sham group. The p-p38, CX3CR and FKN secretion in spinal cord of MCP-1 group were reduced than BCP group as shown in Table 3. The technology for diagnosing and treating tumors has advanced, thereby extending patient life times. Thus, physician's primary focus now is on enhancing the quality of life for patients with advanced cancer. The primary cause of the

reduction in patient's quality of life with advanced cancer is pain from bone cancer, which is brought on by tumor metastasis to bone. Therefore, it is particularly important to reveal the neurobiological mechanism of BCP and explore a safe and efficient new target for the treatment of BCP. At present, it is known that chemokines can act on immune regulation and pain information transmission and regulation, which is an important bridge connecting nerve immune regulation. Spinal cord neurons and glial cells contain many chemokines and their functional receptors. These chemokines and their functional receptors can participate in various pathological pain processes, and their expression in the central nervous system is increased. MCP-1 is a classical chemokine, which can participate in pain information transmission together with its functional receptors[10]. Relevant studies have shown that intrathecal administration of an antibody neutralizing MCP-1 in neuropathic pain model can relieve mechanical hyperalgesia, while intrathecal administration of an antibody neutralizing MCP-1 in normal rats can induce mechanical hyperalgesia, suggesting that MCP-1 has a significant impact in neuropathic pain[11,12]. Clinical studies have shown that the small neurons of MCP-1 in the spinal root ganglia of normal rats can be highly expressed in neuropathic pain models such as spinal nerve ligation[13]. In addition, some scholars found that the expression level of astrocytes in spinal nerve ligation model increased[14]. Other scholars have found that MCP-1 is highly expressed in astrocytes in transgenic mice, and the pain response of mice is exaggerated[15]. In this study, we detected MCP-1 protein in the spinal cord of 6 d, 12 d and 18 d rats, and found that the MCP-1 protein in the spinal cord of 6 d group, 12 d group and 18 d group was higher than sham group. In addition, PWT values of rats were detected. The outcomes demonstrated that PWT values of rats in BCP group were reduced than sham group after 12 d. The PWT value of MCP-1 group was higher than BCP group. It is suggested that the increased MCP-1 in spinal cord of rats with BCP may be closely related to noxious signal transduction, which may play a role in the occurrence and maintenance of BCP. MAPK signaling pathway is relatively conservative in evolution. After activation, MAPK can phosphorylate many nuclear transcription factors and proteases, regulate the transcription of related genes, transfer extracellular stimulation to the nucleus, and finally participate in cell growth and development and play an important role. p38MAPK is an important stress regulatory protein in cells, which can participate in cell signal transduction and gene expression. At present, it has been confirmed that p38MAPK can regulate the process of neural plasticity by regulating cell transcription and protein synthesis. FKN may increase the incidence of an inflammatory cascade reaction and the release of inflammatory mediators, mediate immune damage, and then participate in the onset, progression and play an important role in many diseases. At present, FKN is considered to be involved in the transmission and modulation of nociceptive information. Some studies have found that intrathecal injection of FKN in rats can form hyperalgesia. Pretreatment with CX3CR1 neutralizing antibody or FKN neutralizing antibody and knocking out CX3CR1 receptor gene can inhibit FKN induced hyperalgesia, and improve inflammatory pain and neuropathic pain. Other studies confirmed that intrathecal injection of FKN could activate p38MAPK, and p-p38 was mainly expressed in microglia. After knockout of CX3CR1 gene, the content of p-p38MAPK in activated microglia decreased after peripheral nerve injury. In this study, p-p38, CX3CR1 protein expression levels and FKN secretion in spinal cord of rats were detected. The outcomes demonstrated that the p-p38, CX3C1 and FKN secretion in spinal cord of rats in BCP group were higher than sham group. The p-p38, CX3CR1 and FKN secretion in spinal cord of MCP-1 group were reduced than BCP group.

TABLE 1: COMPARISON OF MCP-1 PROTEIN EXPRESSION IN SPINAL CORD OF RATS ($\bar{x}$±s)

Group	MCP-1
Sham	1.16±0.35
6 d	1.45±0.30*
12 d	1.65±0.27*
18 d	1.75±32*

Note: Compared with sham group, *p<0.05

TABLE 2: COMPARISON OF MECHANICAL PAIN THRESHOLD OF RATS (x̄±s)

Group	0 h	1 h	2 h	4 h	24 h
Sham	15.23±2.23	14.19±1.98	17.53±1.02	16.53±1.58	17.15±1.58
BCP	2.23±0.16*	2.09±0.09*	1.99±0.12*	2.01±0.15*	2.14±0.13
MCP-1	3.15±0.10#	6.89±0.18#	5.16±0.09#	4.59±0.23#	4.02±0.12#

Note: Compared with sham group, *p<0.05 and compared with BCP group, #p<0.05

TABLE 3: COMPARISON OF p-p38, CX3CR PROTEIN EXPRESSION AND FKN SECRETION IN SPINAL CORD OF RATS (x̄±s)

Group	p-p38	CX3CR1	FKN (pg/ml)
Sham	0.21±0.05	0.17±0.03	412.05±114.58
BCP	0.54±0.07*	0.44±0.07*	1395.58±285.48*
MCP-1	0.35±0.06#	0.35±0.04#	582.45±158.29#

Note: Compared with sham group, *p<0.05 and compared with BCP group, #p<0.05

In summary, MCP-1 is up-regulated in rats with advanced BCP. It can regulate FKN secretion by activating p38MAPK signaling pathway, and then act on CX3CR1 receptor and participate in the process of BCP in rats.

Conflict of interests:

The authors declared no conflict of interests.

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