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Persistent pain accelerates xenograft tumor growth of breast cancer in rat

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ABSTRACT

Pain occurs at all stages of the patients who suffer from cancer. Owing to surgery and bone metastasis, breast cancer patients were usually disturbed by persistent pain. However, the pain-relief-right has not been respected enough in clinical cancer treatment. Whether pain has any adverse effects on cancer development is still unclear. In order to uncover this question, we established two preclinical animal models to explore the effects of pain on the tumor. For the first model, we mimicked neuropathic pain by sciatic nerve ligation on rats with xenograft tumor subcutaneously. For the second model, we mimicked the bone cancer pain by injecting tumor cell suspension into the tibial medullary cavity of rats with xenograft tumor subcutaneously. The rats with persistent pain showed higher tumor volume and tumor weight compared with the group without pain. Interestingly, when the neuropathic pain and bone cancer pain were relieved by drug administration, both the tumor volume and tumor weight were lowered compared with the group without pain relief. In summary, our study indicated that persistent pain acted as a contributing factor to tumor growth. Moreover, the pain relief could weakened the accelerating role of pain in tumor growth. Thus, we should be paid more attention to the cancer patients with persistent pain as well as cancer treatment.

1. Introduction

The prevalence of pain is 59% in patients during the process of treatment, 33% after the process of treatment, and more than 60% with advanced patients [1]. Though there are several guidelines for cancer pain management, nearly 50% of patients is undertreated for cancer pain [2]. In addition, the World Health Organization (WHO) estimates that over 80% of the world's population is inadequately treated for moderate to severe pain. Almost all cancer patients encounter pain experiences, which has an adverse impact on survival [3]and quality of life [4].

For example, a prospective study of 86 patients with metastatic breast cancer have described that the survival time of intervention group was longer than the control group, in which one of psychosocial interventions was pain control [5]. Importantly, in another prospective, population-based study, 6331 volunteers with no diagnosis of cancer classified as widespread pain, region pain and no pain. During the follow-up period, those with widespread pain experienced an excess incidence of cancer and reduced cancer survival [6]. Besides, several researchers have investigate the effect of early palliative care on median survival. Compared with standard care group, median survival was nearly 3 months longer in the early palliative care group [7]. Moreover, pain was certified as an independent factor for survival in patients with prostate cancer [8].

Furthermore, pain also had a negative influence on the survival in patients with metastatic breast cancer [9,10]. As early as in 1947, Truscott BM observed that pain was the first symptom in 10% of the 787 breast cancer patients and 16% of them experienced pain before treatment [11]. A recent study showed that over 25% of the 398 breast cancer patients were bothered by pain before the surgery [12]. Nearly 50% of 1543 patients were lived with

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persistent pain after breast cancer surgery in a cross-sectional study [13]. Instead of an outcome of cancer development, pain is closely associated with the procession and the propel malignancy [14].

However, the limited clinical trial data was failure to definitively determine the influence of persistent pain on breast cancer development. We hypothesized that persistent pain as an independent factor has an influence on the progression of breast tumor. In order to prove this hypothesis, we established two tumor-bearing models with persistent pain, including bone cancer pain and neuropathic pain, respectively. Our study was aimed to investigate the influence of pain on breast cancer development.

2. Material and methods

2.1. Animals

Female Wistar rats weighing 160–190 g were kept under the condition maintaining temperature (24 °C ± 0.5 °C), humidity (55% ± 10%) 12 h alternating light-dark cycle light, food and water freely available. The experiment was performed in accordance with the NIH guide for the care and use of laboratory animals and the Ethical Issues of the International Association for the Study of Pain [15].

2.2. Cells

Walker 256 rat mammary gland carcinoma cells (provided by Institute of Acupuncture Research, Fudan University) were derived from Wistar rat. We injected the cancer cell suspension 0.5 ml (1×10^7 cells/ml) into the abdominal cavity of the Wistar rats and collected the ascites after 4–6 days. Then we measured the number of ascites and diluted it to proper concentration.

2.3. Drug administration

For the experiment about the effects of neuropathic pain on tumor growth, rats in CCI+Bupivacaine group (n = 5) and Control Bupivacaine (n = 5) were was received bupivacaine hydrochloride injection (250μl/kg of 0.5% bupivacaine, Harvest Pharmaceutical CO. Shanghai, China) with anesthesia once a day (8:00AM). From the fourth day after tumor inoculation subcutaneously, the administration continued for a week. Meanwhile, The rats of CCI+Saline group (n = 5) were injected with normal saline in the same volume. For the experiment about the effects of bone cancer pain on tumor growth, morphine hydrochloride injection (3mg/kg-1, Northeast Pharm, Shenyang, China) was administrated subcutaneously into the scruff at the back of the neck twice a day (8:00 a.m. and 8:00 p.m.). The rats of the BCP+Morphine group (n = 5) and the Control+Morphine group (n = 4) received twice a day (12 h/12 h) from the fourth day to the ninth day after inoculation. The rats in the BCP+Saline group (n = 5) were injected with normal saline.

2.4. Neuropathic pain mo

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To observe the effect of neuropathic pain on subcutaneous tumor growth, we established the chronic constriction injury of the sciatic never according to the method mentioned by Bennett. GJ and Xie. Y-K [16]. We carried out the anesthesia and made an incision on the skin of middle-thigh. The sciatic nerve was exposed and isolated form the surrounding tissue. Then, three ligatures were tied with 610 surgical suture (1 mm spacing) near the sciatic nerve trigeminal branch. Expect for the never injury, the sham operation group was performed with the same surgery.

2.5. Bone cancer pain model

To observe the effect of bone cancer pain on subcutaneous tumor growth, we established the rat model with Walker 256 mammary gland carcinoma cells [17]. After the anesthetization by sodium pentobarbital (i. p. 40 mg/kg, Sigma, US), 3 mm incisions were made at the skin of the keen joint to expose the tibia plateau. Then we used the 23-gague needle pierced into the tibia cavity about 7 mm and slowly removed. The syringe with Walker 256 carcinoma cells (5×10^4 cells in 15 μl) was injected into the tibia medullary cavity through the same pierce with a 29-gague needle. In order to avoid the overflow, the syringe was remained at the injected site for 3 min. Besides, bone wax was used to seal the pierce in bone. For the sham operation group and the control group, rats was carried out with the same measures, despite the injection with 15 μl normal saline.

2.6. Subcutaneous tumor inoculation with of walker 256 cells

To observe the effect of persistent pain on breast tumor growth in vivo, rats was anesthetized fully and transplanted by injecting 0.5 ml walker256 cell ascites (containing 2×10^6 cells) in the left armpits. At the end of the experiments, we took out the tumors of all rats.

2.7. Von Frey hair test for mechanical allodynia

To test the stable existence of persistent pain, rats were placed in the plexiglass lattice ($26 \times 20 \times 14\ cm^3$) individually and acclimatized for 20 min at the outset of each test. According to the up and down method [18], we used von Frey filaments (Stoelting, Wood Dale, IL, USA) to assay the mechanical sensitivity. Finally, we transformed the record to data and acquired the 50% paw withdrawal mechanical threshold (PWMT) [19].

2.8. Tumor growth measurement

The tumor volumes were recorded using the following formula: $(x \times y^2)/2$, where x and y represent the tumor length and width (in mm), respectively. The tumor weight was acquired after the tumor issue isolated from the rats.

2.9. Statistical analysis

We used SPSS (IBM Corporation, Armonk, NY, USA) version 21.0 for data analyses. All the data were expressed as the mean add standard error of the mean. The mean difference is significant at the 0.05 level. Despite that the tumor volume difference of among Control group, CCI+Saline group and CCI+Bupivacaine group was analyzed by the Kristal–Wallis nonparametric test, as well as that of Control group, BCP+Saline group and BCP+Morphine group. The 50% paw withdrawal mechanical threshold, rat weight and tumor volume were analyzed by the single-factor analysis of variance (one-way ANOVA) test.

3. Results

3.1. Chronic constriction injury of the sciatic serve (CCI) induced persistent neuropathic pain

To determine whether the persistent pain was established, the 50% paw withdrawal mechanical threshold (PWMT) were accessed

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