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ORIGINAL RESEARCH

A Single Intrathecal or Intraperitoneal Injection of CB2 Receptor Agonist Attenuates Bone Cancer Pain and Induces a Time-Dependent Modification of GRK2

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Abstract The objective of this study was to explore the potential role of G-protein-coupled receptor kinase 2 (GRK2) in the progression of cannabinoid 2 receptor (CB2) agonist-induced analgesic effects of bone cancer pain. Female Sprague–Dawley rats, weighing 160–180 g, were utilized to establish a model of bone cancer pain induced by intra-tibia inoculation of Walker 256 mammary gland carcinoma cells. JWH-015, a selective CB2 agonist, was injected intrathecally or intraperitoneally on postoperative day 10. Bone cancer-induced pain behaviors—mechanical allodynia and ambulatory pain—were assessed on postoperative days −1 (baseline), 4, 7, and 10 and at post-treatment hours 2, 6, 24, 48, and 72. The expressions of spinal CB2 and GRK2 protein were detected by Western Blotting on postoperative days −1 (baseline), 4, 7, and 10 and at post-treatment hours 6, 24, and 72. The procedure produced prolonged mechanical allodynia, ambulatory pain, and different changes in spinal CB2 and GRK2 expression levels. Intrathecal or intraperitoneal administration of JWH-015 alleviated the induced mechanical allodynia and ambulatory pain, and inhibited the down-regulation of spinal GRK2 expression. These effects were in a time-dependent manner and reversed by pretreatment of CB2 selective antagonist AM630. The results affirmed CB2 receptor agonists might serve as new treatment targets for bone cancer pain. Moreover, spinal GRK2 was an important regulator of CB2 receptor agonist-analgesia pathway.

Keywords Spinal cord · CB2 · JWH-015 · GRK2 · Bone cancer pain

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Introduction

The incidences of cancer among developing countries are rapidly increasing in recent years (Jemal et al. 2011; Khan et al. 2010). Many epithelial-derived cancers, including breast, prostate, and sarcoma, typically metastasize to bone (DeNardo et al. 2008). Chronic pain, a deleterious effect caused by bone metastases (van den Beuken-van Everdingen et al. 2007), decreases the quality of patients' life and remains a clinical challenge. Therefore, a development of new and effective analgesic therapies with fewer side effects is one of the major goals in bone cancer pain researches. Many types of tumor cells, such as breast cancer, prostate cancer, and sarcoma cells have been

successfully intra-medulla inoculated to establish preclinical pain models (Mao-Ying et al. 2006; Ren et al. 2012; Zhang and Lao 2012). A series of behavioral, neurochemical, and cellular changes are potentially correlated with bone destruction and cancer growth in these models. Since the underlying mechanisms evolve and change with disease progression, all models can reveal the mechanisms that drive bone cancer pain.

In the nervous system, the endocannabinoid signal system based on G-protein-coupled receptors (GPCR), CB1 and CB2 receptors, can mediate the neurotransmission (Fernandez-Ruiz 2009; Marrs et al. 2010). CB1 and CB2 receptors can also modulate many physiological systems, such as memory, mood, and immune (Cabral and Griffin-Thomas 2009; Garcia-Gutierrez et al. 2013; Howlett 1995). Ample evidences suggest that CB2 selective agonists have analgesic effects without psychotropic side effects produced by CB1 receptors agonists in preclinical models (Pertwee 2012; Romero-Sandoval et al. 2008). Furthermore, CB2 receptor agonist displayed the antiallodynic effect in a dose-dependent manner in our previous studies (Gu et al. 2011). Nevertheless, it remains controversial that CB2 receptor agonists could prevent bone loss (Bab et al. 2009; Lozano-Ondoua et al. 2013; Sophocleous et al. 2011). Given that the pharmacological efficacy of CB2 receptor agonists proved complex, it was essential to reveal the underlying molecular mechanisms to fully characterize the therapeutic potential.

GRK2 is a critical modulator of GPCR signaling. GRK2 can phosphorylate GPCRs and dissociate G proteins from binding to the receptor (Benovic et al. 1986; Lohse et al. 1990). Consequently, it causes receptor internalization or desensitization, and thus, the downstream signals are inhibited (Hausdorff et al. 1990; Reiter and Lefkowitz 2006). Generally, the upregulation of receptor signaling is related to a reduction of cellular GRK2 activity (Vroon et al. 2004). Several studies reported the downregulation of spinal GRK2 participated in the modulation of pain signal transmission in different models (Kleibeuker et al. 2007; Wang et al. 2013; Won et al. 2014). However, the role of spinal GRK2 in bone cancer pain remains unclear. In an animal model of neuropathic pain, a blockade of glial (microglia and astrocytes) activation could attenuate neuropathic pain and recover the expression of GRK2. Considerable evidence reports that the IL-1β-activated glia cells are also important regulators of the glial-neuronal GRK2 pathway and essential for the development of chronic pain conditions. Glial-neuronal GRK2 pathways play a critical role in the development of neuropathic pain (Won et al. 2014). Our previous studies have reported that JWH-015, a CB2 receptor selective agonist, could inhibit glial activation and eventually attenuate remifentanil-induced postoperative hyperalgesia (Sun et al. 2014). In addition, JWH-015 produced antinociceptive effects via time-dependent modification of spinal IL-1β and other pro-inflammatory cytokines in bone cancer pain (Lu et al. 2015). Then, we hypothesized that GRK2 protein might be an important regulator of bone cancer pain.

The aim of this study is to investigate whether spinal GRK2 pathway is involved in the development of bone cancer pain and different administration routes of CB2 receptor agonist JWH-015 can exert antinociceptive effects through GRK2.

Materials and Methods

Animals and Design

Female Sprague–Dawley rats (60–80 g body weight for ascites passage; 160–180 g body weight for surgery; Drum Tower Hospital Laboratories, Nanjing, China) were kept under controlled conditions (21 ± 1 °C, 12-h light/dark cycle, food and water ad libitum). According to different experimental conditions, the rats were randomly divided into 5 groups ($n = 8$): (1) sham operated group; (2) tumor-bearing group with intrathecal JWH-015 injection; (3) tumor-bearing group with intrathecal dimethyl sulfoxide (DMSO) injection; (4) tumor-bearing group with intrathecal injection of AM630 30 min before JWH-015; (5) tumor-bearing group with intraperitoneal JWH-015 injection; (6) tumor-bearing group with intraperitoneal DMSO injection; and (7) tumor-bearing group with intraperitoneal injection of AM630 30 min before JWH-015. Behavioral tests were performed on postoperative days −1 (baseline), 4, 7, and 10 and at post-treatment hours 2, 6, 24, 48, and 72. Western blotting analyses were analyzed on postoperative days −1 (baseline), 4, 7, and 10 and at post-treatment hours 6, 24, and 72.

All the experimental procedures used in this study were performed according to the Medical College of Nanjing University Animal Care and Use Committee (Nanjing, China) and in agreement with the National Institutes of Health and the International Association for the Study of Pain (Zimmermann, 1983).

Drugs and Chemicals

JWH-015 and AM630 were both purchased from Sigma (USA), while DMSO was purchased from Amresco (USA). JWH-015 and AM630 were dissolved in DMSO to obtain the final concentration. To determine the effects of peripheral and central CB2 receptors bone cancer pain or GRK2 expression, JWH-015 was injected in two different routes. On postoperative day 10, JWH-015 (10 μg/10 μl) or DMSO (10 μl) was injected into the subarachnoid space through the intervertebral foramen between L5 and L6

(Hylden and Wilcox 1980). JWH-015 (100 μg/500 μl) or DMSO (500 μl) was intraperitoneally injected to another bone cancer group at the same time. To determine whether the effects of JWH-015 were indeed specifically mediated via CB2 receptors, the antagonist AM630 was used. AM630 (15 μg/10 μl, i.t.) and AM630 (100 μg/500 μl, i.p.) were administered 30 min before JWH-015. All solutions were made fresh daily.

Cell Culture

Walker 256 rat mammary gland carcinoma cells (obtained from Shanghai Research Center of Biomedical Engineering, China) were derived from SD rats. A 0.5 ml volume of ascetic cancer cells (2×10^7 cells/ml) was injected into the abdominal cavity of SD rats (60–80 g). The ascitic fluid was extracted 6–7 days after injection followed by centrifugation at 1500 rpm for 5 min. The precipitate was isolated, thrice washed with 10 ml normal saline, and re-centrifuged at 1500 rpm. The precipitate was finally diluted with normal saline to obtain a cell density of 1×10^5/μl using a hemocytometer and maintained on ice until the surgery (Mao-Ying et al. 2006; Zhu et al. 2014).

Anesthesia and Surgical procedure

As previously described (Medhurst et al. 2002; Mao-Ying et al. 2006), an appropriate animal model, which was based on Walker 256 mammary gland carcinoma cells injection in rat tibia and could produce a progressive development of pain, was utilized. In this model, estrous cycle shows no effect on the development of bone cancer pain (Zhu et al. 2014). Rats (160–180 g) were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and placed in a supine position. After the sterile preparation, a longitudinal incision was made through the skin overlying the low third of the left tibia, and thus, the tibia was exposed. A 23-gauge needle was inserted to perforate the bone cortex and 5-μl volume of Walker 256 rat mammary gland carcinoma cells (1×10^5/μl) or normal saline was injected into the intramedullary space using a 25-μl microsyringe. No leakage of cells was spilled outside the tibia. Afterward, the inject eyelet was sealed with bone wax followed by the irrigation with normal saline. The wound was finally closed with 4-0 silk thread (Ethicon, USA) in layers. Rats were placed on a heated pad before the recovery of consciousness and then returned to their home cages. No significant motor impairment was demonstrated in this procedure.

Behavioral Studies

All of the animals were tested in a blind fashion (the experimenters were blind to the treatment groups) during the day portion of the circadian cycle and acclimatized to the test chamber at least 30 min prior to each experiment. The pain behavior test consisted of two tests: ambulatory pain test and mechanical hyperalgesia test.

Ambulatory Pain Assessment

The rats were placed into individual plexiglas observation chamber (50 cm × 50 cm × 40 cm). All animals were allowed to walk across the chamber freely. The extent of ipsilateral limb use was observed for 2 min during spontaneous ambulation and ambulatory pain scores were characterized using the following criteria: score 0, normal use: score 1, slight limp; score 2, limp and guarding behavior; score 3, severe limp or partial non-use of the limb in locomotor activity; and score 4, complete lack of the limb use.

Mechanical Hyperalgesia Assessment

Mechanical hyperalgesia was assessed by Von Frey filaments (Stoelting, Wood Dale, IL, USA). Chaplan's up–down method was chosen (Chaplan et al. 1994). A series of calibrated Von Frey filaments (value ranging from 2 to 15 g) were used to determine the withdrawal thresholds (PWMT) of the paw ipsilateral to the site of surgery. Every Von Frey filament was applied five times and poked vertically to the medial plantar surface for 6–8 s with approximately 10-min interval. The positive pain response was a brisk withdrawal of the hind paw. The lowest Von Frey filament that induced at least three positive pain responses in one trial was considered as the PWMT.

Western Blotting for CB2 and GRK2

The rats were sacrificed with overdose of sevoflurane, and lumbosacral enlargement of spinal cord was immediately removed and stored in liquid nitrogen until further processing. Tissue samples were homogenized in lyses buffer (KeyGEN BioTECH, Nangjing, China). Lysates were centrifuged at 13,000 rpm for 20 min at 4 °C, and supernatants were removed. Protein concentrations in supernatant were determined by BCA Protein Assay Kit. Samples (70 μg) were then separated by SDS-PAGE (10 %) and subsequently transferred onto polyvinylidene fluoride membranes (Pall, USA). Membranes were blocked with 5 % non-fat milk in Tris buffered saline for 1 h at room temperature and followed by incubation with primary antibody for CB2 (1:500; Abcam; ab3561; USA), GRK2 (1:400; Santa cruz; sc-562; USA), or β-actin (1:4000; Abcam; ab52614; USA) at 4 °C overnight. The membranes were then washed forth with PBST and probed with a horseradish peroxidase-coupled secondary antibody

(1:5000; Jackson; 115-005-003; USA) at room temperature for 1–2 h. Finally, these membranes were washed repeatedly with PBST and visualized by chemiluminescence ECL with X-ray film exposure. The density of each band was measured with ImageJ software.

Statistical Analysis

All data are expressed as mean ± SD (standard deviation) and evaluated by SPSS software 17.0. Repeated measurements were performed to determine overall differences in pain behavior changes over time. The effects of treatments upon the expression of GRK2 protein in spinal cord were analyzed by means of one-way analysis of variance (ANOVA), followed by Bonferroni's Multiple Comparison Test. P value <0.05 was accepted as significant.

Results

Bone Cancer Pain Behaviors Induced by Walker 256 Rat Mammary Gland Carcinoma Cells Over Time

As shown in Fig. 1, baseline measurements were similar in different groups. At day 4 after surgery, both sham group and tumor-bearing group rats displayed increased scores of ambulatory pain and decreased PWMT of the ipsilateral hind limb ($P > 0.05$). However, on day 7, the pain behavior values of sham group returned to baseline, while significant differences were in tumor-bearing group ($P < 0.05$). Furthermore, the rats gradually displayed a profound decrease in PWMT and increase in ambulatory pain scores in tumor-bearing group on day 10. ANOVA revealed a significant effect of time (Mechanical hyperalgesia assessment: $F_{3,56} = 17.463$, $P < 0.001$; Ambulatory pain assessment: $F_{3,56} = 30.231$, $P < 0.001$) and a significant time * group interaction (Mechanical hyperalgesia assessment: $F_{3,56} = 18.512$, $P < 0.001$; Ambulatory pain assessment: $F_{3,56} = 30.846$, $P < 0.001$). Taken together, the ambulatory pain and mechanical allodynia were successfully induced by tibia inoculation with Walker 256 rat mammary gland carcinoma cells.

Spinal CB2 and GRK2 Protein Expression During Bone Cancer Pain

To evaluate whether CB2 and GRK2 expression in spinal cord changed along with the ongoing bone cancer pain, Western bolt analysis was performed to quantify CB2 and GRK2 levels on postoperative days −1 (baseline), 4, 7, and 10. Compared with the sham group, CB2 expression in the tumor-bearing group was significantly increased overtime 10 days after surgery. However, the expression of GRK2 decreased in a time-dependent manner after surgery ($F_{3,32} = 19.161$, $P < 0.001$). The decrease started from postoperative day 4 and remained until postoperative day 10 (Fig. 2). Furthermore, there was a significant reduction of spinal GRK2 levels in tumor-bearing group since day 7 after surgery ($P < 0.01$). However, no corresponding changes were observed in the sham group. These data confirmed that spinal GRK2 level was gradually decreasing in the progression of bone cancer pain.

Administration of JWH-015 Alleviated Bone Cancer-Induced Mechanical Allodynia and Ambulatory Pain

To address whether administration of JWH-015 could indeed attenuate bone cancer pain and to determine the antinociceptive effects of intrathecal administration of

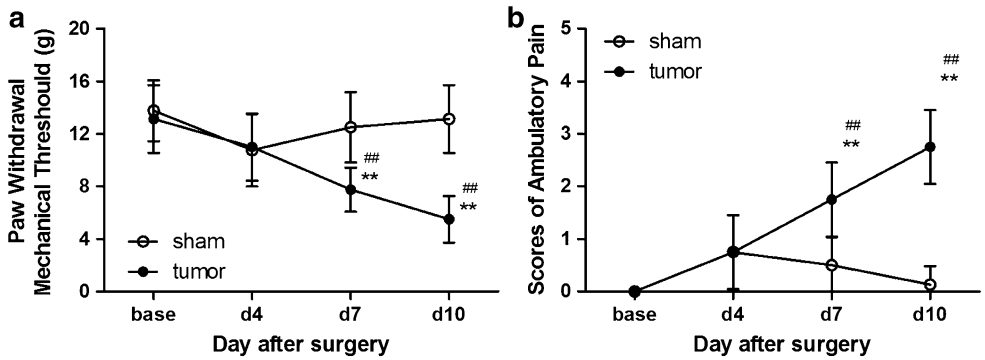

Fig. 1 Development of mechanical allodynia and ambulatory pain following intramedullary injection of Walker 256 rat mammary gland carcinoma cells ($n = 8$). Mechanical allodynia and ambulatory pain were, respectively, evaluated by PWMT and scores of ambulatory pain. PWMT to von Frey filaments (**a**) progressively decreased over time in tumor-bearing group. The scores of ambulatory pain (**b**) progressively increased over time in tumor-bearing group. Significant difference was detected since 7 days after surgery and stable at day 10. Date were presented as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus baseline; $^{*}P < 0.05$, $^{**}P < 0.01$ versus sham group rats

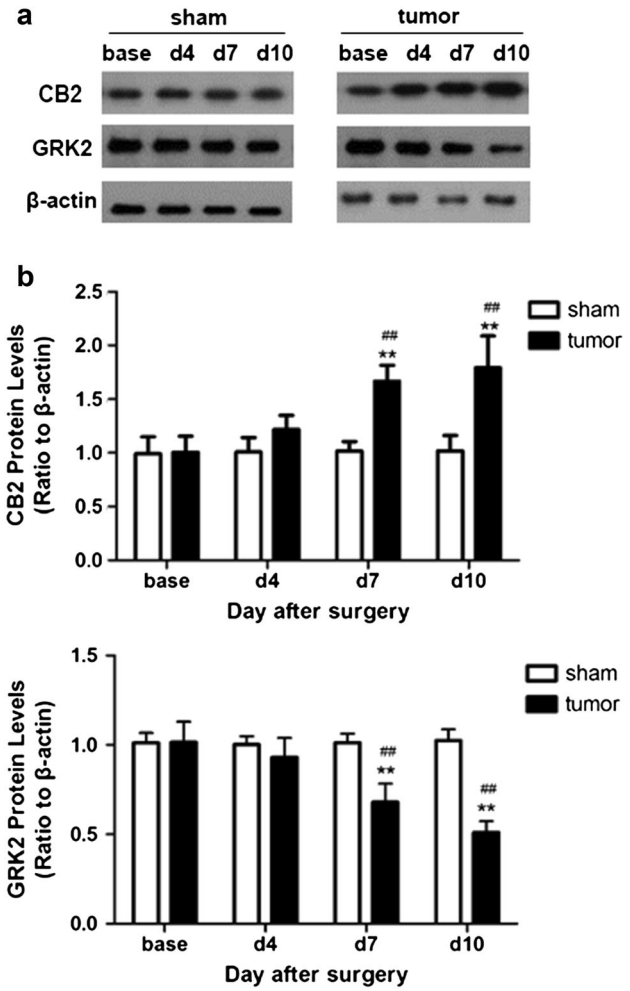

Fig. 2 CB2 and GRK2 expression levels in the spinal cord of sham group and tumor-bearing group ($n = 5$). Western blots (**a**) and statistical analysis (**b**) for CB2 and GRK2 in sham group and tumor-bearing group 4, 7, and 10 days after surgery. CB2 expression was first evident and significantly up-regulated at 7 days after inoculation with Walker 256 rat mammary gland carcinoma cells, and then these values increased over time. GRK2 expression was first evident and significantly downregulated at 7 days after inoculation with Walker 256 rat mammary gland carcinoma cells, and then these values decreased over time. No corresponding changes were observed in the sham group rats. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus baseline; $^{*}P < 0.05$, $^{**}P < 0.01$ versus sham group rats

JWH-015 (10 μg/10 μl) and intraperitoneal administration of JWH-015 (100 μg/500 μl) on bone cancer pain behaviors, we analyzed the pain behavior over time in various groups. On day 10, all animals underwent bone cancer pain, and no significant differences of pain behaviors were shown in different groups. After intrathecal administration of JWH-015, there was an upward tendency of PWMT and a downward tendency of ambulatory pain scores. And there was a significant effect of time (Mechanical hyperalgesia assessment: $F_{8,336} = 258.998$, $P < 0.001$; Ambulatory pain assessment: $F_{8,336} = 167.135$, $P < 0.001$). However, there were no significant differences at 2 h after injection (Fig. 3). Actually, the antinociceptive effect of JWH-015 was beginning at 6 h after injection ($P < 0.05$) and sustained for at least 48 h after injection ($P > 0.05$). Moreover, the analgesia effect of JWH-015 reached its peak at 24 h after injection ($P < 0.01$). As controls, the pain behaviors did not differ in the DMSO treated rats. This result suggested the antinociceptive effect of JWH-015 was a slow-onset and long-duration response in this model. However, the antinociceptive effect of JWH-015 occurred in a reversible manner, as it was reversed in the presence of specific CB2 antagonist AM630 ($P < 0.05$). This was manifested by no significant changes of the PWMT and ambulatory pain scores in AM630 group. When exploring the difference of i.t. and i.p. JWH-015 effect, we found that pain behaviors of JWH-015 (i.p.) group and JWH-015 (i.t.) group were maintained the same trend. Neither AM630 (i.p.) nor DMSO (i.p.) elicited any significant effect on pain behaviors ($P > 0.05$). ANOVA showed a significant time * group interaction (Mechanical hyperalgesia assessment: $F_{40,336} = 4.493$, $P < 0.001$; Ambulatory pain assessment: $F_{40,336} = 6.418$, $P < 0.001$). And at these doses, there were no significant differences between the two groups ($P > 0.05$).

Effect of JWH-015 on Bone Cancer-Induced GRK2 Reduction

To assess the potential effects of JWH-015 on the expression of spinal GRK2, Western bolt was used to analyze the level of spinal GRK2 on postoperative day 10 and at post-treatment hours 6, 24, and 72. As shown in Fig. 4, the expression of GRK2 was inhibited in tumor-bearing group. After intrathecal injection or intraperitoneal injection of JWH-015, the expression of GRK2 was increased at 6 h ($P > 0.05$). This change peaked at 24 h ($P < 0.05$). However, the level of GRK2 at 72 h closely approximated to the level before treatment ($P > 0.05$). This was consistent with the effect on pain behaviors. In contrast, no corresponding changes were exhibited in AM630 and vehicle-treated groups. Both time ($F_{3,96} = 5.754$, $P < 0.001$) and drug effects ($F_{5,96} = 6.223$, $P < 0.001$) were significant, as was the interaction between these factors ($F_{15,96} = 1.918$, $P < 0.05$). These results converged to suggest that the reduction of GRK2 induced by bone cancer pain could be prevented by CB2-selective agonist JWH-015. Furthermore, local or systemic administration of JWH-015 at the doses in this study showed no differences.

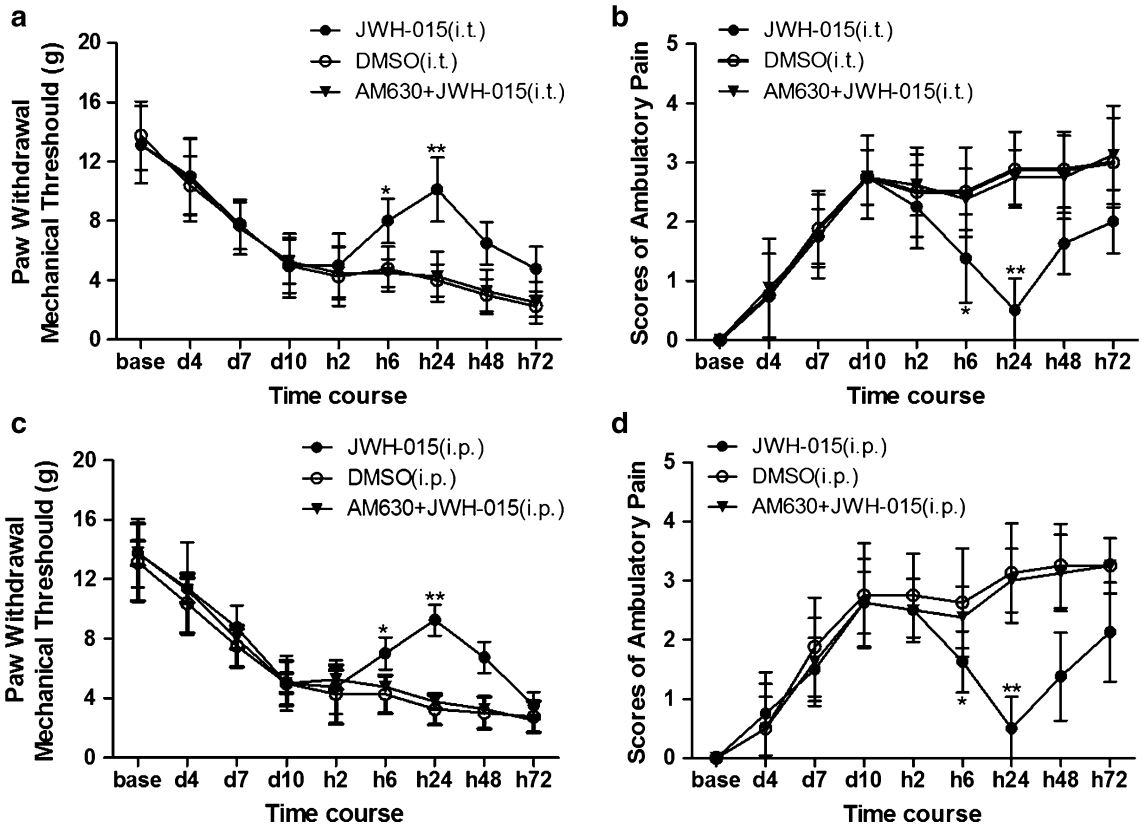

Fig. 3 JWH-015 administration alleviated mechanical hyperalgesia and spontaneous pain in a time-dependent manner ($n = 8$). Pain behaviors were assessed at 1 day before surgery (baseline), 4, 7, and 10 days after surgery and at 2, 6, 24, 48, and 72 h after different treatments. Changes of PWMT (**a**) and ambulatory pain (**b**) in tumor-bearing group after intrathecal injection of JWH-015 (10 μg/10 μl); changes in PWMT (**c**) and ambulatory pain (**d**) in tumor-bearing group after intraperitoneal injection of JWH-015 (100 μg/500 μl). Significant changes were showed at 24 h after JWH-015 administration compared with AM630 or DMSO administration. Each group used eight rats. Date were presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus day 10

Discussion

CB2 receptor selective agonists have been shown analgesic effects in some preclinical models of inflammatory, neuropathic, and bone cancer pain (Romero-Sandoval et al. 2008; Yao et al. 2008). CB2 receptors were previously thought to be restricted to immune system in the periphery (Facci et al. 1995; Howlett et al. 2002). Subsequently, a few CB2 receptors were also presented in CNS regions and with an upregulation in the sites involved in nociception (Atwood and Mackie 2010; Di Marzo 2011). In our previous studies, intrathecal administration of JWH-015 (0.5, 1, and 2 μg) attenuated tumor-evoked tactile allodynia and thermal hyperalgesia in mice, and the effect was dose dependent (Gu et al. 2011). In a rat model, intrathecal injection of JWH-015 (10 μg/10 μl) attenuated remifentanil-induced postoperative hyperalgesia (Sun et al. 2014). So a dose of 10 μg/10 μl of JWH-015 was selected. And combined with domestic and overseas literatures, there is no equivalent dose of JWH-015 by intrathecal or intraperitoneal injection. Then, JWH-015 in an effective dose (100 μg/500 μl) was intraperitoneally injected to another bone cancer group at the same time (Romero-Sandoval and Eisenach 2007; Romero-Sandoval et al. 2008). In the present study, both intrathecal administration and intraperitoneal administration of JWH-015 had analgesic effects and could recover spinal GRK2 expression levels. These results suggested both peripheral and central CB2 receptors contributed to bone cancer pain. Our previous studies displayed that the upregulation of the NR2B subunits of NMDAR contributed to bone cancer pain in mice (Gu et al. 2010), and intrathecal administration of JWH-015 decreased NR2B mRNA expression and attenuated bone cancer pain (Gu et al. 2011). In conclusion, we suggest CB2 receptor agonists may elicit the analgesic effects via various pathways.

In the family of GRKs, which is familiar to regulate homologous desensitization of a wide range of agonist-occupied GPCRs, GRK2 is the most studied member (Lombardi et al. 2002). GRK2 is expressed in all tissues especially in nervous and immune system (Vroon et al. 2006). The desensitization evoked by GRK2 is dependent

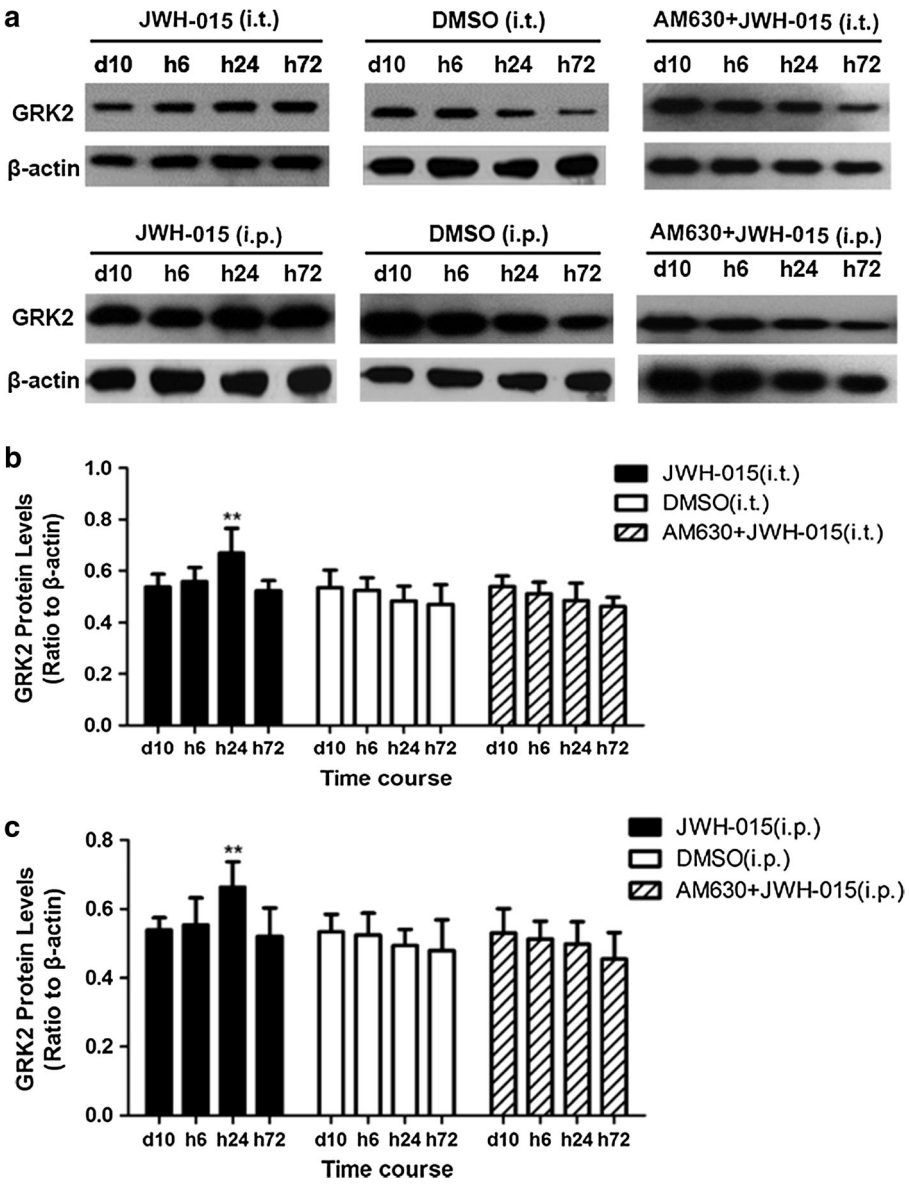

Fig. 4 GRK2 expression after JWH-015, DMSO, and AM630 + JWH-015 administration in tumor-bearing group ($n = 5$). **a** Western blots for GRK2 in spinal cord at different time after different interventions; **b** Changes in GRK2 protein levels in spinal cord of tumor-bearing rats after intrathecal injection of JWH-015 (10 μg/10 μl); **c** Changes in GRK2 protein levels in spinal cord of tumor-bearing rats after intraperitoneal injection of JWH-015(100 μg/500 μl). $^{*}P < 0.05$, $^{**}P < 0.01$ versus day 10

on the phosphorylation of intracellular serine, threonine residues, and the binding of β-arrestins that promote internalization of receptors. This process can prevent cells from overstimulation. Conversely, cells with a reduced expression of GRK2 protein may display prolonged signaling in response to the activation of GPCRs (Homan et al. 2013; Vroon et al. 2006). GRK2 is also revealed to interact with specific downstream intracellular kinases, including Akt, p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) 1/2 (Jimenez-Sainz et al. 2006; Peregrin et al. 2006; Ribas et al. 2007). In addition, GRKs and arrestins might interact with non-GPCR, such as epidermal growth factor (EGF) (Porcile et al. 2004). In vivo, GRK2 protein level in neurons in the spinal horn significantly reduced after neuropathic pain (Kleibeuker et al. 2007, 2008). The increased sensitivity of pain sensing neurons could result in hyperalgesia and allodynia (Woolf and Ma 2007). Our current results also revealed that GRK2 was involved in the progression of Walker 256 rat mammary gland carcinoma cell-induced bone cancer pain. Recently, it was well established that activated microglia and astrocytes released pro-inflammatory cytokines and induced the process of sensitization (Gao and Ji 2009; Kawasaki et al. 2008; Lee et al. 2010). All these could contribute to chronic hyperalgesia in different models of neuropathic pain (Clark et al. 2007; DeLeo and Yezierski 2001; Milligan and Watkins 2009). Considerable evidence suggests that glial-neuronal cross-talk is essential to develop neuropathic pain (Fields and Stevens-Graham 2002; Guo et al. 2007). And low level

of GRK2 facilitated ongoing activation of microglia and astrocytes (Eijkelkamp et al. 2010). Hence, the changes in GRK2 levels contribute to the duration and severity of pain under different neurobiological mechanisms.

In summary, Walker 256 rat mammary gland carcinoma cells could induce bone cancer pain accompanied by the downregulation of GRK2 in the spinal cord of rats. CB2 receptor selective agonist JWH-015 showed antiallodynic effects and recovered spinal GRK2 expression levels. These effects were slow onset but long duration, and there were no significant differences between two routes of administration at certain doses. Combining with compelling evidences that GRK2 degradation could contribute to pain, we suggested CB2 receptor agonists were potentially important new treatment targets for bone cancer pain. Moreover, spinal GRK2 was an important regulator of CB2 receptor-analgesia pathway.

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Authors contributions All of the authors read and approved the final manuscript. CEL made substantial contributions to the experiments. BLH and LYS were mainly involved in the pain behavioral tests. BS and YZ performed the surgical procedure, administration of drugs, and Western blots studies; YES, CEL, and BLH were responsible for statistical analyses. All of these individuals participated in drafting the manuscript. XPG and ZLM conceived the idea, designed the study, and helped revise the manuscript.

Compliance with Ethical Standards

Conflicts of Interest Statement All of the authors declare no conflicts of interest.

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