

2-Bromopalmitate Attenuates Bone Cancer Pain by Reducing Expression Levels of Membrane ASIC3 in Dorsal Root Ganglion Neurons

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Abstract Bone cancer pain (BCP) is one of the most common pains in patients with malignant cancers, and greatly affects the quality of patient life. Understanding the mechanism and improving novel agents for BCP treatment is desirable. As an inhibitor of protein palmitoylation, 2-bromopalmitate (2-BP) improved pain behaviors in neuropathic pain model, whereas its function on BCP was unclear. Acid-sensing ion channel 3 (ASIC3) plays an important role in various pain conditions. However, whether ASIC3 expression was regulated by 2-BP was unknown. In order to investigate the effect of 2-BP on BCP and detect the regulation of 2-BP on ASIC3, we did these research. First, we established BCP rats model through intrathecally injecting rat mammary gland carcinoma cells (MRMT-1) into the left tibia of Sprague-Dawley female rats. On post-operative day (POD) 21, tibia radiographs of BCP rats showed regional bone destruction and damage. And mechanical pain sensitivities of BCP rats were significantly increased compared with sham rats (Sham PWT *vs* BCP PWT: 16.1 ± 1.5 *vs* 5.3 ± 1.5 ; $P < 0.01$), indicating that BCP rats model was working. Second, compared with normal and sham rats, ASIC3 expression in L4-L6 DRG neurons of BCP rats was significantly increased (0.63 ± 0.03 , 0.64 ± 0.1 and 1.07 ± 0.05). Third, compared to the vehicle-treated BCP rats, 2-BP treated BCP rats resulted in a significant increase in PWT (at post injection 6 h, PWT vehicle *vs* PWT 2-BP: 6.9 ± 2.0 *vs* 10.8 ± 1.6 , $P < 0.01$), indicating 2-BP treatment had an antinociceptive effect in BCP rats. Forth, after 2-BP treated, membrane ACIS3 (M-ASIC3) expression in DRG of BCP rats decreased compared with that before 2-BP treated (1.05 ± 0.13 , 0.66 ± 0.12). And in ASIC3-mediated acidosis pain model, 2-BP treatment decreased the number of flinches (vehicle treated rats: 27 ± 1.8 , 2-BP treated rats: 10 ± 1.5), indicating 2-BP treatment blocked ASIC3-mediated acidosis pain. Fifth, the levels of M-ASIC3 were reduced after treatment with 2-BP in ASIC3-transfected SH-SY5Y cells (1.0 ± 0.2 , 0.58 ± 0.10). These data illustrate that 2-BP attenuated bone cancer pain by reducing membrane ASIC3 expression levels in DRG neurons.

Key words bone cancer pain (BCP); 2-bromopalmitate (BP); dorsal root ganglion (DRG); acid-sensing ion channels 3 (ASIC3)

2-溴棕榈酸调节背根神经节中 ASIC3 蛋白的膜定位 缓解大鼠骨癌痛

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摘要 骨癌痛(BCP)是恶性肿瘤患者最常见的疼痛之一,严重影响患者的生活质量。BCP 的分子作用机制和新药研发都迫在眉睫。2-溴棕榈酸(2-BP)作为一种蛋白质棕榈化抑制剂在病理性疼痛中有镇痛效果,而在骨癌痛中作用仍不清楚。酸敏感离子通道 3 型(ASIC3),作为一个重要的疼痛因子能否受到 2-BP 的调控也未知。为了检测 2-BP 在骨癌痛中的作用,并研究其对背根神经节(DRG)中 ASIC3 的调控,本文开展了相关工作。1)首先建立 BCP 大鼠模型,将大鼠乳腺癌细胞(MRMT-1)注射入雌大鼠胫骨髓腔内,21 d 后通过 X 射线和机械痛检测,发现与假性手术组相比,BCP 模型大鼠的胫骨被破坏;同时,BCP 组大鼠的机械疼痛值明显上升(假性手术组 PWT *vs.* BCP PWT:16.1 ± 1.5 *vs.* 5.3 ± 1.5; *P* < 0.01);表明大鼠乳腺癌骨转移疼痛模型成功构建。2)蛋白质免疫印迹检测结果显示,与正常和假性手术组相比,BCP 大鼠 L4-L6 DRG 中酸敏感离子通道 3 蛋白表达上调(0.63 ± 0.03, 0.64 ± 0.1 和 1.07 ± 0.05)。3)在术后第 21 d,给 BCP 大鼠腹腔注射 2-BP,发现给药组 BCP 大鼠的机械疼痛值下调(6 h 后,PWT 对照 *vs.* PWT 2-BP: 6.9 ± 2.0 *vs.* 10.8 ± 1.6, *P* < 0.01),表明 2-BP 在骨癌痛模型大鼠中具有镇痛作用。4)蛋白质免疫印迹结果显示,与给药前相比,2-BP 处理后降低了 BCP 大鼠 L4-L6 DRG 中膜上 ASIC3 蛋白的表达(1.05 ± 0.13, 0.66 ± 0.12)。同时,在 ASIC3 介导的酸痛模型中,2-BP 给药降低大鼠震颤的次数(对照组为 27 ± 1.8 次,2-BP 组为 10 ± 1.5 次),表明 2-BP 给药阻断 ASIC3 介导的酸痛。5)在 ASIC3 转染的 SH-SY5Y 细胞中,与对照相比,2-BP 给药后明显降低膜上 ASIC3 蛋白表达量(1.0 ± 0.2, 0.58 ± 0.10)。这些结果表明,2-BP 在骨癌痛中具有镇痛作用,其镇痛机制涉及到调控背根神经节中膜上酸敏感离子通道 3 的表达。

关键词 骨癌痛;2-溴棕榈酸;背根神经节;酸敏感离子通道 3 型

中图分类号 R741.02

Cancer pain is one of the most common clinical symptoms associated with malignant cancers^[1]. Up to 50% of malignant cancers will eventually metastasize to bone. Once tumor cells begin to grow in the bone, a cycle including tumor growth, bone destruction and formation of woven bone will begin, resulting in significant bone pain^[2]. Bone cancer pain (BCP) is dull and constant BCP increases with time and greatly affects the quality of patient life and their functional states^[2, 3]. Until now, the pathological and molecular mechanisms of bone cancer pain have not been fully elucidated. Meanwhile, existing pharmacological treatments for BCP are not uniformly effective and have undesirable side effects. Then, understanding the mechanism and finding novel agents for treating BCP is desirable.

Bone cancer induces mechanical bone deformation and local tissue acidosis which activates acid-sensitive nociceptors, particularly transient receptor acid-sensing ion channel 3 (ASIC3)^[4]. During bone cancer, proton is released, decreasing extracellular pH level (pH 4.0-5.0) and maintaining an acidic pH microenvironment at the osteoclast-mineralize bone interface^[5]. These released protons activate ASIC3 expression in both dorsal root ganglion (DRG) cell bodies and sensory terminals, which contributes to proton-evoked pain signaling^[6]. ASIC3 plays an important role in various pain conditions such as inflammatory pain^[7], postoperative pain^[8],

migraine^[9] and bone cancer pain^[10-12].

Protein palmitoylation, the covalent lipid modification of the side chain of cysteine residues with the 16-carbon fatty acid palmitate, plays important roles in the regulation of protein targeting to membranes and synapses^[13]. 2-bromopalmitate (2-BP) is a non-metabolizable protein palmitoylation inhibitor, which blocks palmitate incorporation onto proteins and affects the function of proteins^[14]. In synapses, palmitoylation regulates NMDAR (N-methyl-D-aspartate receptors) and AMPA receptors trafficking and function^[15]. In chronic dorsal root ganglia compression (CCD) rat model which presented neuropathic pain, 2-BP treatment markedly improved pain behaviors and down-regulated the expression of NMDAR palmitoylation in spinal level^[16]. Nevertheless, the effects of 2-BP on ASIC3 in DRG were still unclear. In this study, we investigated the antinociceptive effects of 2-BP in BCP rats and examined the expression levels of membrane ASIC3.

1 Materials and Methods

1.1 Animals and 2-BP treatment

Animals used for bone cancer model and all experiments were female Sprague-Dawley (SD) rats weighing 160-200 g. All of them were purchased from the Experimental Animal Center of Hubei Province (Wuhan, China). All experiments conformed to local and international guidelines on ethical use of animals

and all efforts were made to minimize the number of animals used and their suffering.

2-bromopalmitate (2-BP) (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) (Sigma, USA). In animal experiments, 2-bromopalmitate was diluted with saline to a final DMSO concentration of 10%, and then injected intraperitoneally in BCP rats at 1 mg/kg/100 μ L. In cell experiments, 2-BP was diluted with DMSO in different concentrations.

1.2 Preparation of cells

MRMT-1 rat mammary gland carcinoma cells were purchased from JENNIO Biological Technology (China). Cells were cultured in medium containing RPMI 1640 (GIBCO), 10% (50 mL) fetal bovine serum (FBS, heat-inactivated) (Hyclone), 1% (5 mL) L-glutamine and 2% (10 mL) penicillin/streptomycin (GIBCO). Cells were collected by brief exposure to 0.1% w/v trypsin, then washed twice and suspended in an appropriate volume of Hank's solution to achieve final concentration (4×10^6 /mL). Hank's solution was also used for inoculation in sham rats.

SH-SY5Y neuroblastoma cells were cultured in DMEM (GIBCO) containing 10% fetal bovine serum (Hyclone), 50 U/mL penicillin and 50 mg/mL streptomycin (GIBCO). Cells were grown in a humidified incubator at 37°C with 5% CO₂. The plasmid GFP-ASIC3 was a gift from Xu TL^[17]. In cell experiments, cells were transiently transfected with GFP-ASIC3 (4 μ g) and eGFP-C1 (4 μ g) as a control using Lipofectamine © 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Cells were collected by brief exposure to 0.1% w/v trypsin, then washed twice and collected for Western blot.

1.3 Rat formalin test

Rats were acclimated to the observation cage for 30 minutes before testing. Totally 50 μ L formalin (5% formalin in 0.9% saline) was injected into the dorsal side of the left hind paw, and the rat was immediately placed in transparent polycarbonate box. The number of formalin-injected paw flinches in 1-minutes epochs, and measurements were taken at 5-minutes intervals beginning immediately after formalin injection and ending 60 minutes later.

1.4 Rat model of BCP

A rat model of BCP was established following previous reports^[18]. In brief, female SD rats were deeply anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneal injection). The left leg of the rat was shaved and the top half of the tibia was carefully exposed after disinfection with 7% iodine and 75% (V/V) ethanol. MRMT-1 cells (10 μ L, 3×10^4) were slowly injected into the intramedullary space of the left tibia in the BCP rats, whereas an equivalent volume of Hank's solution was injected into the sham

rats using a 10 μ L Hamilton microsyringe. The syringe was left in the injection site for an additional minute to prevent the leakage of tumor cells. The injection site was sealed with bone wax after the syringe was removed. To evaluate the tumor growth, the tibia was visualized radiographically.

1.5 Mechanical Allodynia

Rats were placed on a 5 \times 5-mm wire mesh grid floor and allowed to habituate for 30 minutes. The von Frey filaments (Stoelting, Wood Dale, IL, USA) (ranging from 0.4 g to 26 g) were used to apply mechanical stimuli to the left hind paw. The 50% paw withdrawal threshold (PWT) was determined by Chaplan's up-down method^[19]. In brief, the calibrated monofilaments were applied perpendicularly to the plantar surfaces until the filaments were bent, and a brisk withdrawal was considered as positive response, whenever a positive response occurred. The von Frey filament with the next lower force was applied to test whenever a negative response occurred. Then the filament with the next higher force was applied. The pattern of positive and negative withdrawal responses was converted to 50% PWT.

1.6 Western blot

Ipsilateral L4-L6 DRGs were collected and frozen in lipid nitrogen. Frozen DRGs and cells were homogenized in a RIPA lysis buffer containing a cocktail of protease inhibitors (Sigma). After centrifugation at 12 000 g for 15 minutes, the supernatant was used for Western blotting analysis. Equal amounts of protein samples were separated in 10% SDS-PAGE. The protein was transferred onto a PVDF membrane and then incubated with the appropriate primary antibodies including anti-ASIC3 (abcam, ab49333), β -actin (Sigma, A1978) overnight. HRP-conjugated secondary antibodies (1: 5 000, Jackson Lab) were used to visualize the primary antibodies. Infrared Imaging System (Gene Company Limited, Hong Kong, China) were applied to detect immunoreactive bands.

1.7 MTT assay

MTT were used to test the effects of 2-BP on cell viability. The cells of 5×10^4 SH-SY5Y were seeded in 96-well micro-culture plates in 100 μ L medium containing 0 – 0.3 mmol/L 2-BP for cell viability assays. After incubation for 48 hours, cells were washed with PBS buffer and new medium containing 10% (V/V) fetal bovine serum and MTT was added to give a final concentration of 0.5 mg/mL and incubated for a further 4 hours. The medium was replaced with 150 μ L of pure dimethyl sulfoxide and the absorbance of the dark blue formazan was measured with a microplate reader at 492 nm. Data on cell viability with error bars were expressed as mean \pm SD of 4 independent experiments.

1.8 Statistical analysis

Comparison of values between different experimental groups was done using one-way analysis of variance (one-way ANOVA) with repeated measures followed by Bonferroni post hoc tests. Significance was ascribed for $P < 0.05$. All raw data are presented as mean \pm SEM.

2 Results

2.1 Intra-tibia MRMT-1 cell injection induced bone destruction and mechanical allodynia

In this study, we used female SD rats injected with intra-tibia MRMT-1 rat mammary gland carcinoma cells as animal model for bone cancer pain (BCP).

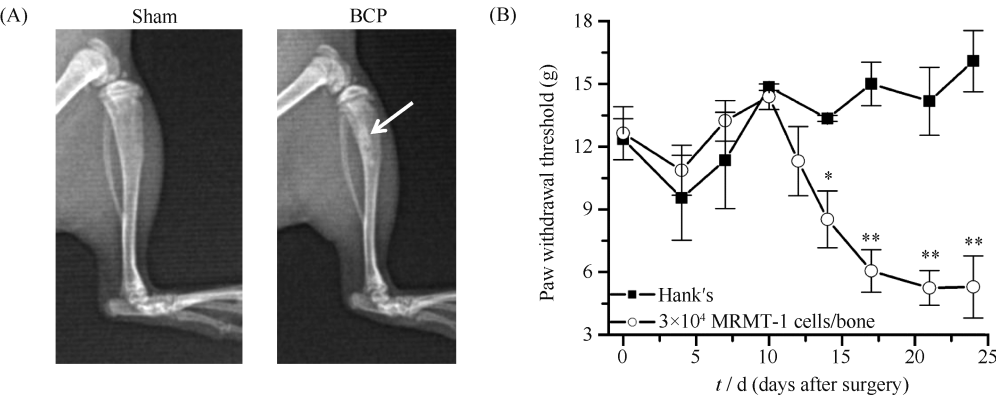


Fig.1 Intra-tibia MRMT-1 cell injection induced bone destruction and mechanical allodynia in BCP rats MRMT-1 cells were injected into the intramedullary space of the left tibia in BCP rats. (A) On 21 day after surgery, X-ray examination demonstrated bone destruction in BCP rats (right), and no changes in sham rats (left). (B) Mechanical PWT showed that BCP rats were mechanical allodynia. PWT was tested using a series of calibrated von Frey filaments prior to tumor cell inoculation (0 day) and at post-operative day (POD) 4, 7, 10, 14, 17, 21 and 24. ■: Sham group, ○: Model group. All data were expressed as the mean \pm SEM, $n=9$. * $P < 0.05$, ** $P < 0.01$ vs the Hank's group rats at each corresponding time point

2.2 ASIC3 expression was significantly increased in L4-L6 DRG neurons of BCP rats

To check the roles of ASIC3 in BCP, we examined the expression of ASIC3 in L4-L6 DRG neurons in normal, sham and BCP rats. As shown in Fig. 2A and B, ASIC3 expression in L4-L6 DRG neurons of BCP rats was significantly increased as

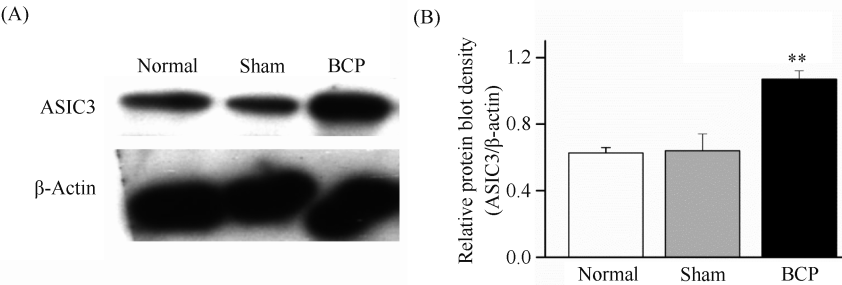


Fig.2 Expression of ASIC3 was increased in L4-L6 DRG neurons of BCP rats (A) Western blot analysis of ASIC3 protein level in the ipsi-lateral L4-L6 DRG neurons of normal, sham and BCP rats on POD 21. (B) Image J software was used to quantify the gray degree values. The results were shown as the mean \pm SD. Three independent experiments were performed. ** $P < 0.01$ compared with the value of ASIC3/β-actin in normal and sham group rats

As a result, tibia radiographs of BCP rats on post-operative day (POD) 21 showed regional bone destruction and damage, whereas there was no significantly change for that of sham rats (Fig. 1A). As shown in Fig. 1B, in BCP rats receiving intra-tibia tumor cell injection, mechanical pain sensitivities were significantly increased, beginning on POD 14 (Sham PWT vs. BCP PWT: 13.3 ± 0.2 vs. 8.5 ± 1.4 ; $P < 0.05$), increasing through POD 17 (Sham PWT vs BCP PWT: 15.0 ± 1.0 vs. 6.1 ± 1.0 ; $P < 0.01$), and continuing through POD 24 (last time point examined; Sham PWT vs BCP PWT: 16.1 ± 1.5 vs. 5.3 ± 1.5 ; $P < 0.01$). Nevertheless, no significant changes in pain sensitivity were detected in sham rats.

compared with normal and sham rats. The value of ASIC3/β-actin in normal, sham and BCP rats were 0.63 ± 0.03 , 0.64 ± 0.1 and 1.07 ± 0.05 , respectively.

2.3 Treatment with 2-BP reversed pain behavior in BCP rats

To investigated the roles of 2-BP in BCP, we

injected intraperitoneally vehicle or 1 mg/kg 2-BP into BCP rats on POD 21 and detected the PWT changes. As shown in Fig.3A, there was no major difference for PWT changes before and after vehicle injection. Compared to the vehicle-treated group, 2-BP treated group resulted in a significant increase in PWT at 0.5 hour (PWT vehicle *vs* PWT 2-BP: 5.1 ± 0.8 *vs* 10.1 ± 1.0 , $P < 0.05$), 2 hours (PWT vehicle *vs* PWT 2-BP: 6.2 ± 2.0 *vs* $10.1 \pm$

1.7, $P < 0.05$), 4 hours (PWT vehicle *vs* PWT 2-BP: 5.5 ± 1.4 *vs* 9.6 ± 1.6 , $P < 0.05$), and 6 hours (PWT vehicle *vs* PWT 2-BP: 6.9 ± 2.0 *vs* 10.8 ± 1.6 , $P < 0.01$) post injection. However, 2-BP had no effect on PWT of normal rats (Fig. 3B). 2-BP treatment increased PWT of BCP rats, indicating that 2-BP treatment reversed pain behavior and 2-BP had an antinociceptive effect in BCP rats.

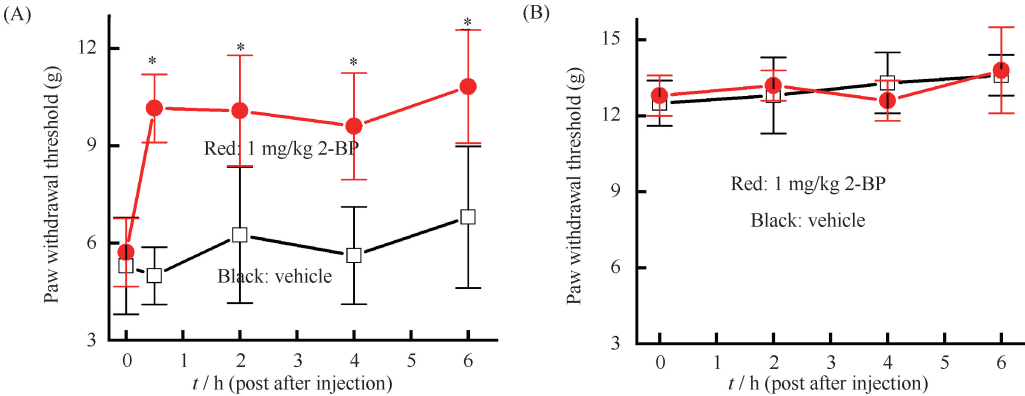


Fig.3 2-BP treatment increased PWT of BCP rats (A) PWT of BCP rats on POD 21 after intraperitoneal injection of vehicle (DMSO + saline) or 1 mg/kg 2-BP. Black□; vehicle treated BCP rats; Red●; 1 mg/kg 2-BP treated BCP rats. All data were expressed as the mean ± SEM, $n = 9$. * $P < 0.05$ *vs.* the vehicle group at each corresponding time point. (B) PWT of normal rats after intraperitoneal injection of vehicle (DMSO + saline) or 1 mg/kg 2-BP. Black□; vehicle treated normal rats; Red●; 1 mg/kg 2-BP treated normal group. All data were expressed as the mean ± SEM, $n = 9$

2.4 2-BP treatment decreased membrane ASIC3 expression in L4-L6 DRG neurons of BCP rats and blocked ASIC3-mediated acidosis pain

To detect the effects of 2-BP on membrane ASIC3 (M-ASIC3) expression, 1 mg/kg 2-BP was intraperitoneal injected into BCP rats on POD 21. After 6 hours, L4-L6 DRG neurons were dissected out and prepared for Western blotting. We detected M-ASIC3 expression in L4-L6 DRG neurons of BCP rats before

and after 2-BP treatment. As shown in Fig.4A, after 2-BP treatment, membrane ACIS3 expression decreased compared with that before 2-BP treated. The values for M-ASIC3/ β -actin before and after 2-BP treated were 1.05 ± 0.13 , 0.66 ± 0.12 , respectively (Fig. 4B). Further, we examined the effects of 2-BP on the ASIC3-mediated acidosis pain which was potently blocked by amiloride, an ASIC channel blocker^[20]. As shown in Fig.4C, the number

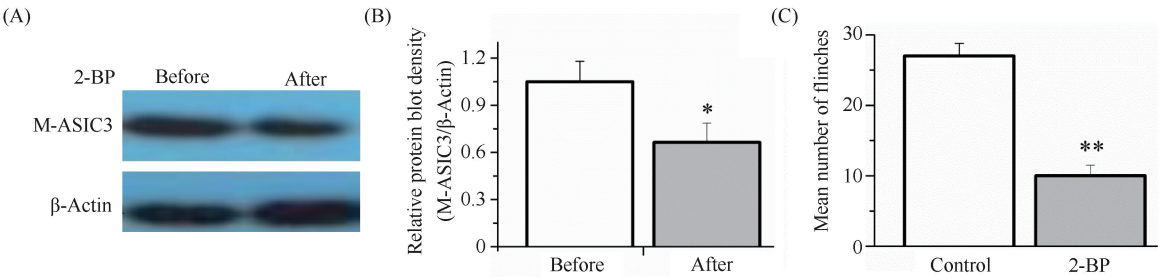


Fig.4 2-BP treatment decreased membrane ASIC3 expression in L4-L6 DRG neurons of BCP rats and blocked ASIC3-mediated acidosis pain (A) Western blot analysis of M-ASIC3 protein level in L4-L6 DRG neurons of BCP rats on POD 21 before and after 2-BP treated. (B) Image J software was used to quantify the gray degree values. Results were shown as the mean ± SD. Three independent experiments were performed. * $P < 0.05$ compared with the value of M-ASIC3/ β -actin before 2-BP treated BCP rats. (C) 2-BP treatment blocked ASIC3-mediated acidosis pain. Control group rats were intraperitoneal injected with vehicle (DMSO + saline), and 2-BP group rats were intraperitoneal injected with 1 mg/kg 2-BP. Flinching shaking of paw was recorded as the number of flinches per observation period (5 minutes). ** $P < 0.01$ compared with the number of flinches in control conditions, unpaired *t*-test, compared with control column. $n = 9$

of flinches decreased from 27 ± 1.8 of control conditions to 10 ± 1.5 with 2-BP treatment ($n=9$, $P<0.01$), suggesting that 2-BP blocked the ASIC3-mediated pain. These data illustrated that expression and function of ASIC3 were regulated by 2-BP, and ASIC3 was involved in the antinociceptive effect of 2-BP in BCP rats.

2.5 2-BP regulated expression levels of membrane ASIC3 in transfected SHSY5Y cells

To verify the regulation of 2-BP on ASIC3 protein, SHSY5Y cells were transfected with GFP-ASIC3 and then treated with 2-BP. First, we tested the effects of 2-BP treatment on toxicity of SH-SY5Y neuroblastoma cells using MTT reduction assay.

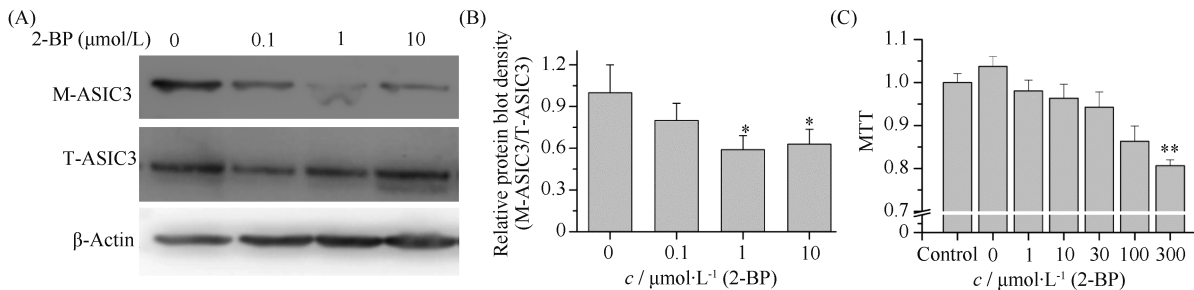


Fig. 5 2-BP decreased expression levels of membrane ASIC3 in ASIC3-transfected SH-SY5Y cells (A) Western blot analysis of GFP-ASIC3 protein level in membrane (M-ASIC3) and total lysis (T-ASIC3) of SH-SY5Y cell treated with 0, 0.1, 1 and 10 $\mu\text{mol/L}$ 2-BP for 24 hours. (B) Image J software was used to quantify the gray values. The results were shown as the mean \pm SD. Three independent experiments were performed, * $P<0.05$. (C) Cell toxicity of 2-BP on the SH-SY5Y cells. The effects of different concentration of 2-BP on the cell viability of SH-SY5Y were determined by MTT reduction assay. Data were expressed as the mean \pm SD, $n=5$, ** $P<0.01$

3 Discussions

3.1 2-BP has antinociceptive effects in pain

2-BP is a non-metabolisable palmitate analogue, and has become deeply investigated in the palmitoylation field. It is often referenced as the pharmacological tool to block protein palmitoylation^[21]. Other groups had demonstrated that 2-BP reversed the pain behavior in a chronic dorsal root ganglia compression (CCD) rat model^[16]. Our present study showed that 2-BP also has antinociceptive effect in BCP rats.

3.2 Protein palmitoylation was involved in 2-BP antinociceptive effect

The sensation of bone cancer pain includes peripheral and central sensitization. In peripheral sensitization, cancer induces mechanical bone deformation and local tissue acidosis, and activates acid-sensitive nociceptors, transient receptor potential vanilloid receptor type 1 (TRPV1) and ASIC3 in DRG^[22]. Here, we just focused on ASIC3, and found that 2-BP treatment regulated M-ASIC3 expression in BCP rats and transfected cells. The effect of 2-BP on TRPV1 was still unclear. Stimulus of the nociceptors in

Treatment with 0-10 $\mu\text{mol/L}$ of 2-BP had no obvious toxicity in the SH-SY5Y neuroblastoma cells (Fig. 5C). The expression level of GFP-ASIC3 was assayed by Western blot analysis. As shown in Fig. 5A and B, the values of membrane GFP-ASIC3 (M-ASIC3)/total lysis ASIC3 (T-ASIC3) treated with 0, 0.1, 1 and 10 $\mu\text{mol/L}$ of 2-BP were 1.0 ± 0.2 , 0.81 ± 0.12 , 0.58 ± 0.10 and 0.63 ± 0.98 , respectively. The levels of M-ASIC3 were reduced after treatment with 1 and 10 $\mu\text{mol/L}$ 2-BP ($P<0.05$). The GFP-ASIC3 protein level in total lysis (T-ASIC3) was not changed. These data indicated that 2-BP decreased the expression levels of membrane ASIC3 in ASIC3-transfected SH-SY5Y cells.

the periphery leads to central sensitization in central nervous system (spinal cord and brain). NMDAR was involved in the initiation of central sensitization. It is reported that spinal NR2B (subunit of NMDAR) was palmitoylated in CCD rats, and 2-BP treatment down-regulated NR2B (subunit of NMDAR) palmitoylation and modulated NR2B phosphorylation which mediated the function of NMDAR^[16]. The above information suggested that 2-BP inhibited ASIC3 protein palmitoylation, and regulated the localization of ASIC3.

3.3 2-BP had an anti-inflammatory effect in pain

During bone cancer pain, inflammatory signal pathway was activated and a lot of inflammatory mediators were increased and released^[22]. It was reported that 2-BP had an anti-inflammatory effect by blocking myristoylation and palmitoylation of Fyn (Src family kinases), inhibiting its membrane binding and localization in T-cell signal pathway^[23]. In order to test the anti-inflammatory effects of 2-BP in pain, we used the inflammatory rat model of pain induced by formalin injection. As shown in Fig. 6, in vehicle treated group of formalin pain rats, the late phase (20-45 minutes) was inflammatory pain. In 2-BP treated

group, the inflammatory pain was inhibited, indicating that 2-BP had an anti-inflammatory effect in pain. The mechanisms behind the anti-inflammatory effect need further study.

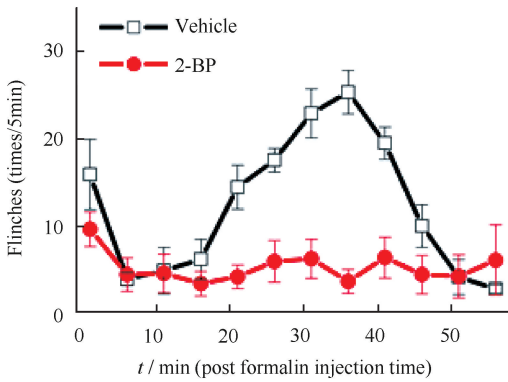


Fig. 6 2-BP treatment has an anti-inflammatory effect in formalin pain rat model The flinch/shaking response of rats in the formalin pain after intraperitoneal injection of vehicle (DMSO + saline) or 2-BP. Flinching shaking of paw was recorded as the number of flinches per observation period (5 minutes)

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