Original Research

Preconditioning with hydrogen sulfide prevents bone cancer pain in rats through a proliferator-activated receptor gamma/p38/Jun N-terminal kinase pathway

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Impact statement

Bone cancer pain (BCP) significantly decreases the life quality of patients or their life expectancy and causes a severe health burden to the society. However, as the exact mechanism of BCP is still poorly understood, no effective treatment has been developed yet. There are some pain medicines now, but they have some inevitable side effects. Additional therapeutic strategies are urgently needed. First, we revealed that preconditioning with H_2S significantly reduced BCP, demonstrated by the decrease of thermal hyperalgesia and mechanical allodynia. Second, the mechanism of H_2S preconditioning was elucidated. It may involve microglia deactivation and inflammation inhibition in the spinal cord, in which the proliferator-activated receptor gamma/p38/Jun N-terminal kinase pathway is activated. This novel finding may significantly help us to understand the difference between the roles of endogenous H_2S and exogenous H_2S in the development of BCP and present us a new strategy of pain management.

Abstract

Bone cancer pain (BCP) is a severe type of hyperpathic pain occurring with primary bone tumors or advanced cancers which metastasize to bones. BCP can detrimentally reduce quality of life and presents a challenge to modern medicine. Studies have shown that exogenous H_2S may act as a neuroprotectant to protect against some diseases in central nervous system. The preset study aimed to investigate the antinociceptive effect of H_2S in BCP. We first measured the changes of serum H_2S in patients with BCP and analyzed the relationship between them, then investigated the effect of H_2S preconditioning on BCP, and explored the mechanism in rat model. Our results revealed that serum H_2S level was negatively correlated with pain scores. In the rat model of BCP, preconditioning with H_2S significantly reduced BCP, demonstrated by the decrease of thermal hyperalgesia and mechanical allodynia. The mechanism of H_2S preconditioning may involve microglia deactivation and inflammation inhibition in the spinal cord, in which the proliferator-activated receptor gamma/p38/Jun N-terminal kinase pathway is activated.

Keywords: Bone cancer pain, hydrogen sulfide, preconditioning, microglia, proliferator-activated receptor gamma/p38/Jun N-terminal kinase pathway, inflammatory cytokines

Experimental Biology and Medicine **2018; 243: 57–65. DOI: 10.1177/1535370217740859**

Introduction

BCP is a severe type of hyperpathic pain which can be induced by both primary bone tumor and advanced cancer when it metastasizes to bones.[1] The human skeleton is the most common target of metastatic cancer, which induces neuropathic pain that usually requires radiotherapy, hypercalcemia, or even spinal cord/nerve root compression to get relief. The unique mechanism of BCP makes it resistant to morphine treatment and the most unbearable symptom accompanying primary bone cancers and bone metastases.[2] As a result, BCP significantly

decreases the life quality of patients or their life expectancy and causes a severe health burden to the society.[3] As the exact mechanism of BCP is still poorly understood, no effective treatment has been developed yet. There are some pain medicines now, but they have some inevitable side effects. Opiate pain relievers may induce constipation, drowsiness, dizziness, lightheadedness, or feeling faint, or even addiction when they are taken for more than a few days. Non-steroidal anti-inflammatory drugs, on the other hand, may cause stomach upset, heartburn, stomach ulcers, and kidney injury if used for a long duration.[4] As the complicated mechanism involved in BCP is still unclear, the development of pain management approaches is challenging, which makes additional therapeutic strategies urgently needed.[5]

H_2S has been recognized as a hazardous gas for a long time. Recent studies found that H_2S could be synthesized via cystathionine beta-synthase (CBS) in mammalian tissues. It is involved in many physiological and pathological processes, including neurotransmission or inflammation.[6] Also, it has been reported that exogenous H_2S may act as a neuroprotectant to protect against many central nervous system (CNS) diseases[7] by its antioxidation, anti-inflammation, and antiapoptosis ability in models of many disorders in the CNS.[8–10] A recent study investigated the pain-relieving profile of some H_2S donors, including natural allyl-isothiocyanate, synthetics phenyl- and carboxyphenyl-isothiocyanate in animal models of neuropathic pain. The results showed that single subcutaneous administrations of H_2S donors and prototypical H_2S donor NaHS reduced the hypersensitivity to cold non-noxious stimuli. The antineuropathic properties were abolished by the H_2S-binding molecule hemoglobin, suggesting the anti-neuropathic role of H_2S.[11] However, the effect of H_2S preconditioning on neuropathic pain was seldom studied.

Some studies have implied that microglial activation and inflammatory cytokines in the nervous system might be essential in the regulation of neuropathic pain.[12,13] Pottabathini *et al.* found out that peroxisome proliferator-activated receptor gamma (PPARγ) has got tremendous importance in nerve trauma and pain.[14] His results revealed that treatment with PPARγ agonist pioglitazone significantly prevented the behavioral, biochemical, mitochondrial, and cellular alterations in spinal nerve ligated rats.[14] Multiple studies have shown the critical role of p38 mitogen-activated protein kinases (MAPK)/Jun N-terminal kinase (JNK) pathway in the neuropathic pain. Zhang *et al.*[15] suggested that activation of β2-adrenergic receptor in the spinal cord reduced neuropathic pain by reducing phosphorylation of microglial p38 MAPK and astrocytic JNK pathway.

Hence, in the present study, to study the antinociceptive effect of H_2S in BCP, we first measured the changes of plasma H_2S in patients with BCP and analyzed the relationship between them, then tested the hypothesis that pretreatment of H_2S prevents BCP in rats. Hyperalgesia, allodynia, and inflammatory cytokines levels in the spinal cord were examined. The roles of PPARγ, p38 MAPK, JNK, ERK, and microglia and astrocyte were also investigated to elucidate the underlying mechanism.

Materials and methods

Patient general information

A total of 45 patients with painful bone metastasis at Yunnan Tumor Hospital were included in this study, including 22 males and 23 females, aged from 43 to 60 years. They received patient's pain self-evaluation Visual Analogue Scales for three consecutive days and got the mean pain score, similarly to Cong *et al.*[16] All of them received standard treatment of pain. A total of 10 healthy individuals were recruited into Control group. The clinical trials were conducted according to Declaration of Helsinki principles, approved by the ethics committee of Yunnan Tumor Hospital. Inclusion criteria and Exclusion criteria are as same as the study of Cong *et al.*[16]

Measurement of serum H_2S levels

Measurement of serum H_2S levels was similar to the studies of Tian *et al.*[17] Venous blood (5 mL) was collected from patients at 10:00 each day for three consecutive days and centrifuged at 3000 r/min for 10 min. Standard sulfion and antioxidant solutions were prepared and a sulfur electrode in PXS-270 ion meter provided by Leici Company (Shanghai, China) was used to measure the H_2S levels in the plasma. The electrode was activated in deionized water, then the ion meter was adjusted to mV; the rake ratio was adjusted to 100. The sulfur and reference electrodes were used to get reading and calculate the H_2S concentration.

Animals and treatment

Adult male Wistar rats (230–250 g) were purchased from Yunnan Tumor Hospital experimental animal center and housed in the animal center of Yunnan Tumor Hospital. The room was lighted from 07:00 until 19:00. Rats were provided with food and water *ad libitum*. The whole experimental protocol was carried out under the guidelines of the International Association for the Study of Pain[18] and was approved by the Animal Care and Use Committee of Yunnan Tumor Hospital.

H_2S inhalation

Similarly to Kida *et al.*,[19] rats were put into a 30 L plastic chamber and allowed to breath air or air–H_2S mixture at room pressure for seven days. Rats breathed H_2S from 09:00 to 17:00 on each day. The H_2S gas/air mixture continually flows through the chamber so the H_2S concentration was maintained at 40 ppm. After the H_2S inhalation, rats rested for 24 h prior to BCP procedure. Serum H_2S levels in rats were measured immediately after the H_2S inhalation with the method described above.

BCP procedure

Rats underwent BCP procedure according to the method of Mao-Ying *et al.*[20] Briefly, the mammary gland carcinoma cells were first prepared from Wistar rat. After rats were anaesthetized with ketamine (100 mg/kg, i.m.) and xylazine (7.5 mg/kg, i.m.), carcinoma cells (4×10^5) or

heat-killed carcinoma cells (sham group) were injected into the medullary cavity of tibia in 6 μL PBS with a 23-gauge needle. The wound was closed after the needle was removed 2 min later. Rats received i.m. injections of ampicillin and meloxicam for prophylaxis of postsurgical infection. All animals were allowed to three-day recovery before the following tests.

Assessment of thermal hyperalgesia and mechanical allodynia

The assessment of hyperalgesia and allodynia was similar to Yao *et al.*[21] For thermal hyperalgesia test, paw withdrawal latencies to a noxious thermal stimulus were recorded using a paw thermal stimulator (IITC Inc., Woodland Hills, CA, USA). Temperature was controlled at 30°C to make the baseline latency be 10–12 s. The average time to withdraw their paws from the thermal stimulus of rats was recorded as the "paw withdrawal latency" (repeated for three times). To prevent tissue damage, the maximum time was set at 20 s. For mechanical allodynia test, von Frey test was applied on the sciatic innervation surface of the hind paws. If rats respond positively to some filament for two times, the weight of the filament was recorded. The tests were carried out every other day to allow the rats to rest and keep a normal response to the stimulation. All the tests were performed by a professional investigator who was blind to the experimental design between 10:00 and 11:00.

Western blot analysis

The western blot analysis was similar to Hulse *et al.*[22] Briefly, rats were first terminally anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused with cold saline solution. Afterward, the lumbar region of the spinal cord was collected and homogenized at 1000 *g* for 15 min. The proteins from supernatant were run on a SDS-PAGE gel (90 V, 1 h 30 min) and transferred to nitrocellulose membrane (100 V, 1 h). Membranes were then incubated with primary antibodies of p-PPARγ, PPARγ, p-p38, p38, p-ERK, ERK, p-JNK, JNK, Iba-1, GFAP, and β-actin overnight at 4°C. All primary antibodies were brought from Cell Signaling Technology (Danvers, MA, USA). After that, the membranes were washed and incubated with secondary antibody (1:2000; Santa Cruz, USA) at 25°C for 60 min. The band intensity was analyzed with the Quantity One software (Bio-Rad, Hercules, USA).

Evaluation of inflammatory cytokines (IL-1β, IL-6, and TNF-α)

The lumbar region of the spinal cord was collected after rats were sacrificed. After they were homogenized at 1500 *g* for 15 min, the supernatant was collected for inflammatory cytokines assays. The concentration of IL-1β, IL-6, and TNF-α was determined with commercial ELISA assays (DuoSet kits, R&D Systems, USA), following the instructions supplied by the manufacturer. The results are expressed as pg/mg protein.

Statistical analysis

All data are presented as the mean ± standard error of the mean. Pearson's correlation was used to detect and analyze the correlation between serum H_2S levels and pain scores. Data in the animal experiments were analyzed by one-way analysis of variance followed by Bonferroni *post hoc* test using SPSS 17.0. The criterion for statistical significance was $P < 0.05$.

Results

Correlation between serum H_2S levels and pain scores

To establish the correlation between serum H_2S levels and pain scores in BCP patients, we analyzed the results with Pearson's correlation. As shown in Figure 1, the serum H_2S level was negatively correlated with pain scores. When the serum H_2S level gets higher, the pain score gets lower. The statistical analysis showed that the correlation is significant ($y = -0.102x + 10$, $R^2 = 0.590$, $r = -0.768$, $P < 0.0001$). This result demonstrated that high levels of H_2S in patients have low pain score, indicating a possible antinociceptive effect of H_2S.

H_2S preconditioning increased the serum H_2S level in rats

The serum H_2S level in rats was measured immediately after the H_2S/air inhalation was done in four groups ($n = 10$ for each group). As illustrated in Figure 2, the normal serum H_2S level in rats in the Sham and BCP rats was around 0.2 μM. Daily preconditioning with H_2S for seven days increased the serum H_2S level in rats to around 2.2 μM in BCP + H_2S and H_2S groups ($P < 0.05$).

H_2S preconditioning improved thermal hyperalgesia and mechanical allodynia induced by BCP

As shown in Figure 3, daily preconditioning with H_2S for seven days reduced thermal hyperalgesia and mechanical allodynia induced by BCP procedure. There is a significant decrease in thermal withdrawal latency (Figure 3(a)) and mechanical withdrawal threshold (Figure 3(c)) in BCP group, which were prevented by H_2S preconditioning

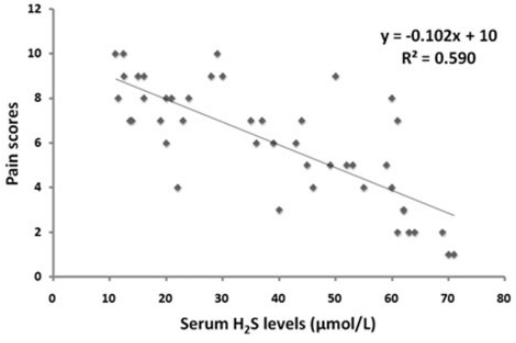

Figure 1. Relationship between serum H_2S levels and pain scores in bone cancer pain (BCP) patients. The result showed the serum H_2S levels were negatively correlated with pain scores. The statistical analysis showed that the correlation is significant ($y = -0.102x + 10$, $R^2 = 0.590$, $r = -0.768$, $p < 0.0001$).

($P < 0.05$). The thermal hyperalgesia or mechanical allodynia was not changed in the contralateral side of BCP rats (Figure 3(b) and (d)). H_2S preconditioning alone did not affect withdrawal latency or mechanical withdrawal threshold in rats (Figure 3(a) to (d)).

H_2S preconditioning activated PPARγ, p38, and JNK in the spinal cord, but not ERK

The western blot analysis showed that BCP caused a slight increase in p-PPARγ, p-p38, and p-JNK expression. When rats were treated with H_2S preconditioning, they were further increased (Figure 4(a) to (c)). BCP or H_2S preconditioning caused no changes in p-ERK expression. When rats were treated with H_2S preconditioning alone, the expression of p-PPARγ, p-p38, p-JNK, or p-ERK was not significantly altered.

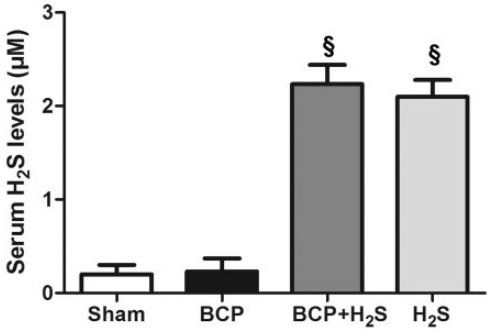

Figure 2. H_2S preconditioning increased the serum H_2S level in rats. The serum H_2S level in rats was measured immediately after the H_2S/air inhalation. The normal serum H_2S level in the Sham and BCP rats was around 0.2 μM, while the serum H_2S level in BCP + H_2S and H_2S groups is around 2.2 μM. Values are expressed as mean ± standard error of the mean (SEM). §: $P < 0.05$ compared to BCP. N = 10 per group.
BCP: bone cancer pain.

H_2S preconditioning decreased the expression of inflammatory cytokines in spinal cord

To investigate the role of H_2S preconditioning on inflammation, TNF-α, IL-1β, and IL-6 levels in the spinal cord were measured by ELISA. As shown in Figure 5(a) to (c), BCP treatment sharply increased the inflammatory cytokines in the spinal cord, while H_2S preconditioning significantly decreased them ($P < 0.05$). No significant change in the TNF-α, IL-1β, and IL-6 levels was found when rats were treated with H_2S preconditioning alone.

H_2S preconditioning attenuated the spinal microglia activation caused by BCP

As shown in Figure 6(a), BCP increased the expression of Iba-1 (microglia marker) located in the ipsilateral dorsal horn, which was significantly reduced by H_2S preconditioning ($P < 0.05$). Figure 6(b) showed that GFAP (astrocyte marker) was not changed by BCP or H_2S preconditioning.

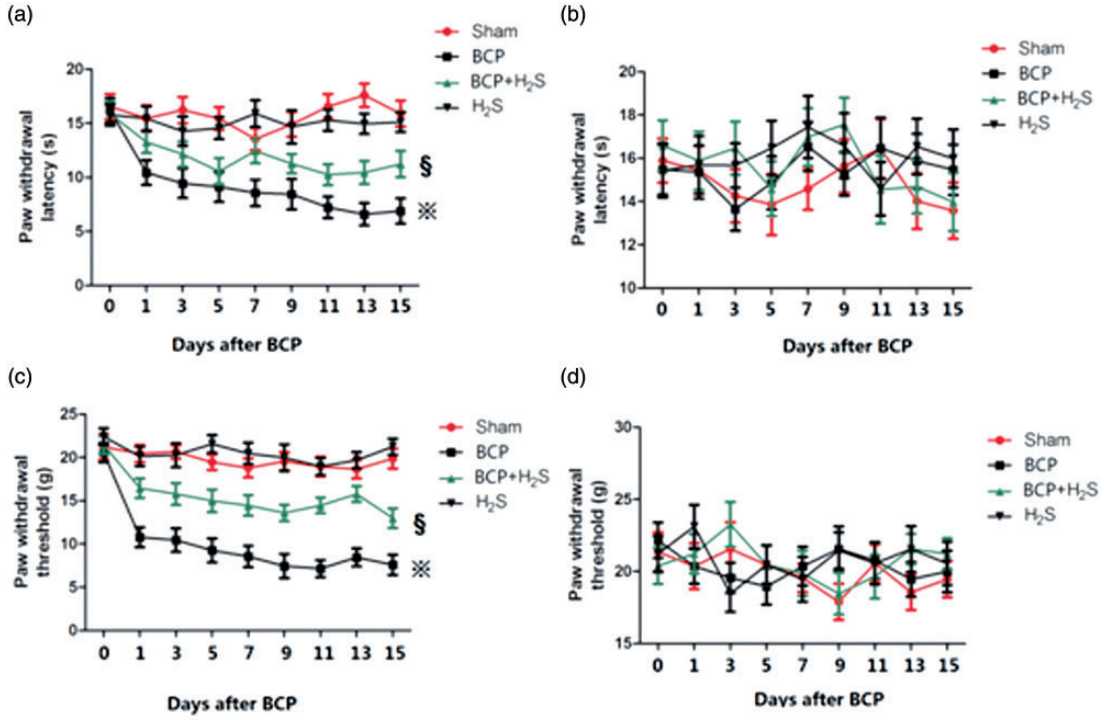

Figure 3. H_2S preconditioning improved thermal hyperalgesia and mechanical allodynia. A sharp decrease of thermal withdrawal latency (a) and mechanical withdrawal threshold (c) was observed in the groups of rats treated with BCP, which were prevented by H_2S preconditioning. (a) Thermal hyperalgesia (ipsilateral), (b) thermal hyperalgesia (contralateral), (c) mechanical allodynia (ipsilateral), and (d) mechanical allodynia (contralateral). Values are expressed as mean ± SEM. ※: $P < 0.05$ compared to Sham; §: $P < 0.05$ compared to BCP. N = 10 per group. BCP: bone cancer pain. (A color version of this figure is available in the online journal.)

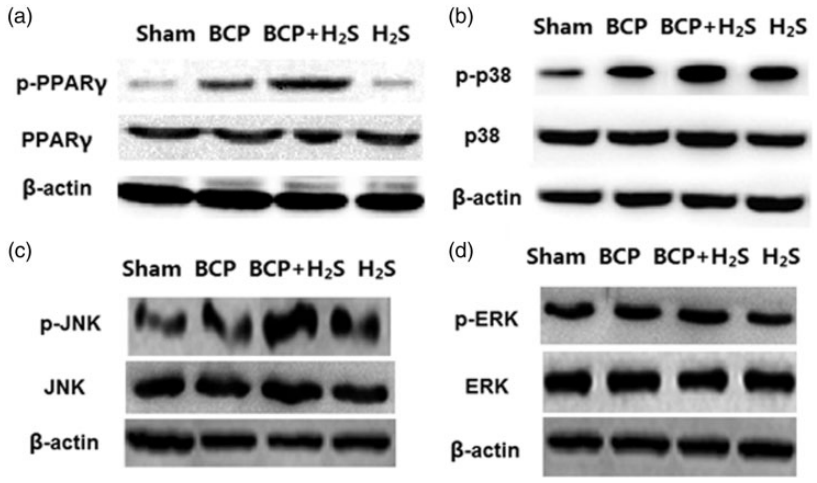

Figure 4. H_2S preconditioning activated PPARγ, p38, and JNK in the spinal cord. BCP resulted in a slight increase in p-PPARγ, p-p38, and p-JNK expression (a–c), which was further increased by H_2S preconditioning. N = 10 per group.
BCP: bone cancer pain; ERK: extracellular signal regulated kinase; JNK: Jun N-terminal kinase; PPARγ: proliferator-activated receptor gamma.

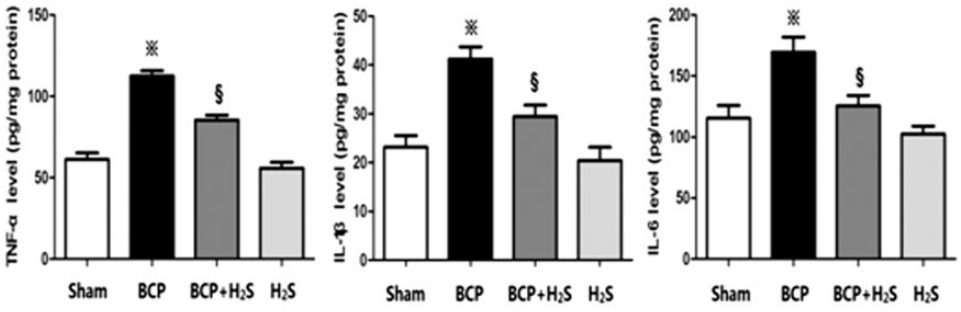

Figure 5. H_2S preconditioning decreased the expression of inflammatory cytokines (TNF-α, IL-1β, and IL-6) in spinal cord. BCP treatment sharply increased the expression of TNF-α, IL-1β, and IL-6 in the spinal cord, while H_2S preconditioning significantly decreased them. Values are expressed as mean ±SEM. ※: $P < 0.05$ compared to Sham; §: $P < 0.05$ compared to BCP. N = 10 per group. BCP: bone cancer pain; IL-1β: Interleukin-1beta; IL-6: Interleukin-6; TNF-α: tumor necrosis factor-alpha.

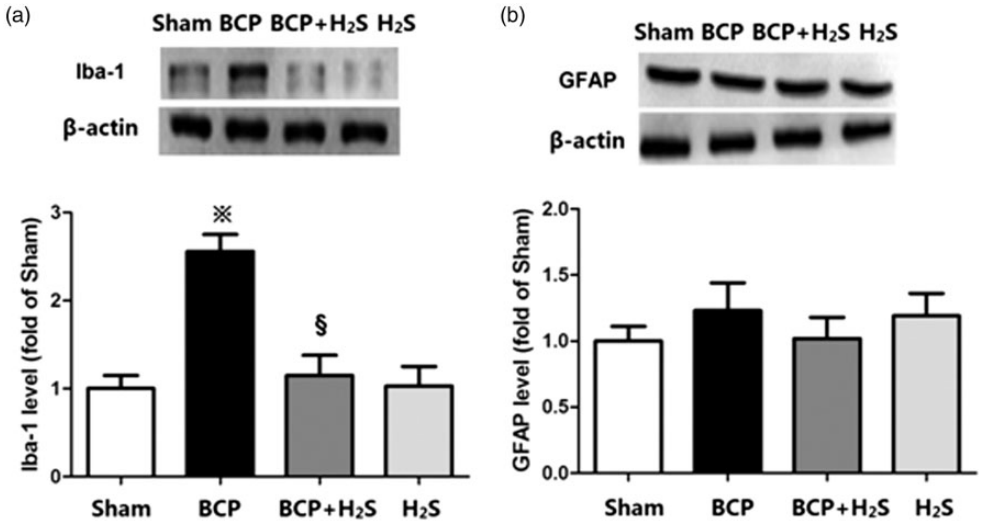

Figure 6. H_2S preconditioning attenuated spinal microglia activation. BCP resulted in an increase of Iba-1 (microglia marker) expression in the ipsilateral dorsal horn of the lumbar spinal cord, while H_2S preconditioning significantly reduced this Iba-1 expression in the ipsilateral dorsal horn. GFAP (astrocyte marker) was not changed. Values are expressed as mean ±SEM. ※: $P < 0.05$ compared to Sham; §: $P < 0.05$ compared to BCP. N = 10 per group.
BCP: bone cancer pain; Iba-1: ionized calcium-binding adapter molecule 1; GFAP: glial fibrillary acidic protein.

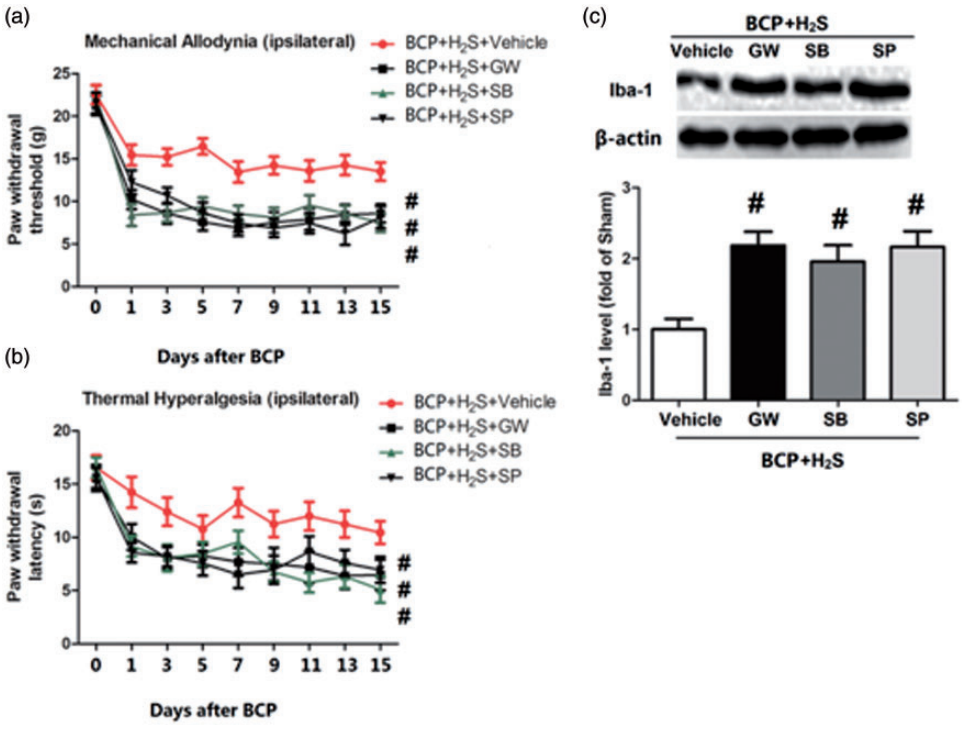

Figure 7. Inhibitors of PPARγ/p38/JNK pathway abolished the effects of H_2S preconditioning on thermal hyperalgesia and mechanical allodynia and microglia activation. PPARγ inhibitor GW 9662, p38 inhibitor SB203580, and JNK inhibitor SP600125 reversed the effects of H_2S preconditioning on thermal hyperalgesia and mechanical allodynia and microglia activation. Values are expressed as mean ±SEM. #: $P < 0.05$ compared to BCP + H_2S. N = 10 per group. BCP: bone cancer pain; GW: GW 9662; Iba-1: ionized calcium-binding adapter molecule 1; SB: SB203580; SP: SP600125. (A color version of this figure is available in the online journal.)

Inhibitors of PPARγ/p38/JNK pathway abolished the effects of H_2S preconditioning on thermal hyperalgesia and mechanical allodynia and microglia activation

To explore the involvement of PPARγ/p38/JNK pathway, we treated rats with PPARγ inhibitor GW 9662, p38 inhibitor SB203580, and JNK inhibitor SP600125, then remeasured the effects of H_2S preconditioning on thermal hyperalgesia and mechanical allodynia as well as microglia activation. The results showed that after rats were given GW 9662, SB203580, and SP600125, thermal hyperalgesia and mechanical allodynia were significantly decreased compared to the BCP+ H_2S group (Figure 7(a) and (b)). These inhibitors also increased microglia activation, as demonstrated by the increased expression of Iba-1(Figure 7(c), $P < 0.05$). GW 9662, SB203580, and SP600125 alone did not affect the pain threshold (data not shown).

Inhibitors of PPARγ/p38/JNK pathway abolished the effects of H_2S preconditioning on inflammatory cytokines in spinal cord

To explore the role of PPARγ/p38/JNK pathway on inflammatory cytokines, we treated rats with PPARγ inhibitor GW 9662, p38 inhibitor SB203580, and JNK inhibitor SP600125, then remeasured the effects of H_2S preconditioning on inflammatory cytokines. As shown in Figure 8(a) to (c), after rats were given GW 9662, SB203580, and SP600125, the expression of TNF-α, IL-1β, and IL-6 in the spinal cord was increased compared to the BCP+ H_2S group ($P < 0.05$).

Discussion

High concentration of H_2S was considered a toxic gas which could inhibit complex IV in the electron transport chain.[23] In recent years, accumulating scientific evidence has shown that H_2S is a third inorganic gaseous mediator (the other two are nitric oxide and carbon monoxide). Low concentration of H_2S has protective effects on various injuries.[9,24–28] For the first time, we demonstrated that in the BCP patients, the serum H_2S level was negatively correlated with pain scores. The Pearson's statistical analysis showed that the correlation is significant ($r = -0.768$, $p < 0.0001$), indicating that H_2S may be closely correlated with the pathology of BCP.

Preconditioning with H_2S has raised special attention from scientists as it can evoke numerous downstream signaling pathways, such as Akt-GSK-3β signaling.[29,30] However, the majority of the research subjects are ischemia/reperfusion injuries. The effect of H_2S preconditioning on BCP has been less studied. The effect of H_2S preconditioning on neuropathic pain has been less studied. Several studies have revealed the relationship between neuropathic pain and H_2S (mostly endogenous H_2S) and the results are controversial. Lin *et al.*[31] found out that NaHS, a H_2S donor, alleviated chronic neuropathic pain by inhibiting expression of p-cAMP response element binding in the spinal

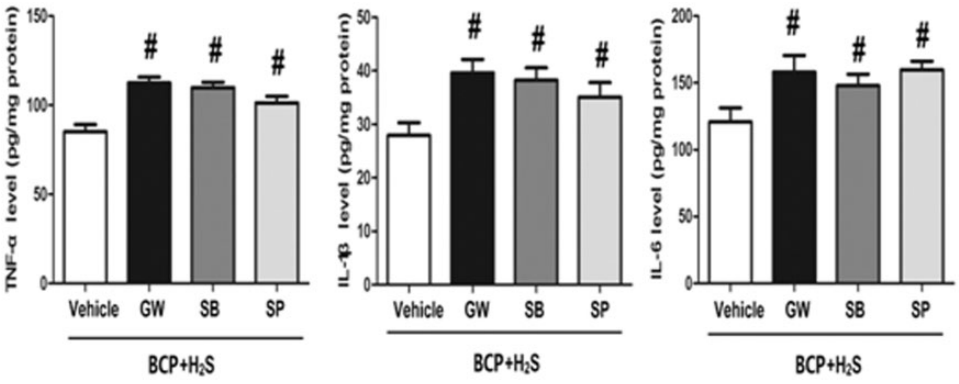

Figure 8. Inhibitors of PPARγ/p38/JNK pathway abolished the effects of H_2S preconditioning on inflammatory cytokines (TNF-α, IL-1β, and IL-6) in spinal cord. PPARγ inhibitor GW 9662, p38 inhibitor SB203580, and JNK inhibitor SP600125 reversed the effects of H_2S preconditioning on TNF-α, IL-1β, and IL-6 levels. Values are expressed as mean ±SEM. #: $P < 0.05$ compared to BCP + H_2S. N = 10 per group.
BCP: bone cancer pain; GW: GW 9662; IL-1β: ; IL-6: ; SB: SB203580; SP: SP600125; TNF-α: .

cord. In another study, sensitization of purinergic P2X3 receptors was found to be mediated by CBS–H_2S signaling in primary sensory neurons and contribute to discogenic pain.[32] Similarly, Terada and Kawabata[33] suggested that H_2S appeared to facilitate the functions of anticalcium channel Cav3.2 and transient receptor potential cation channel A1. Also, endogenous CBS/H_2S pathway was proven to promote the development of neuropathic pain.[34] To further study the mechanism of H_2S involved in BCP, we preconditioned rats with H_2S in an animal model of BCP. Our study confirmed that daily preconditioning with H_2S for seven days increased the serum H_2S level in rats from 0.2 μM to around 2.2 μM. H_2S pretreatment significantly relieved neuropathic pain induced by BCP, as demonstrated by the decrease in thermal hyperalgesia and mechanical allodynia. As shown in Figure 3, daily preconditioning with H_2S for seven days prevented the development of thermal hyperalgesia and mechanical allodynia. The sharp decrease of thermal withdrawal latency and mechanical withdrawal threshold induced by BCP were prevented by H_2S preconditioning.

Qu *et al.*[35] demonstrated that inhibitors of MAPKs could reduce mechanical allodynia and MAPKs-positive neurons in dorsal root ganglia play an important role in the mechanism. More particularly, the study of Zhou *et al.*[36] revealed that paeoniflorin and albiflorin could inhibit the p38 MAPK pathway and subsequently up-regulated proinflammatory cytokines (IL-1β and TNF-α). Similarly, PPARγ prevents neuropathic pain development in rats, while blockade of PPARγ with GW9662 reversed the inhibitory effect of pioglitazone on hypersensitivity.[37] In the present study, we found that BCP resulted in a slight increase in p-PPARγ, p-p38, and p-JNK expression, which was further increased by H_2S preconditioning. BCP treatment sharply increased the expression of TNF-α, IL-1β, and IL-6 in the spinal cord, which were significantly decreased by H_2S preconditioning. These results suggest that H_2S preconditioning may regulate the PPARγ/p38/JNK pathway and inhibit the inflammatory response and BCP.

Microglias are immunocompetent cells in the CNS. They can quickly respond to injury and play an important role in maintaining tissue homeostasis. However, under abnormal conditions, including severe traumatic injury or inflammation in the CNS, microglia may secrete excess proinflammatory cytokines and reactive oxygen species, which exacerbate the neuronal damage.[38] Recently, microglia has emerged as a key player in eliciting neuropathic pain in the spinal cord.[10,39] The fact that BCP increased Iba-1 (microglia marker) expression in the dorsal horn indicates the pivotal role of microglia in BCP development. The fact that H_2S preconditioning decreased the Iba-1 expression suggests that the suppression of microglia proliferation by H_2S preconditioning may be an important mechanism of the antinociceptive effects of H_2S preconditioning.

To further confirm the involvement of PPARγ/p38/JNK pathway, we treated rat with inhibitors of PPARγ/p38/JNK pathway and then remeasured the thermal hyperalgesia and mechanical allodynia and microglia activation, as well as inflammatory cytokines. The results showed that PPARγ inhibitor GW9662, p38 inhibitor SB203580, or JNK inhibitor SP600125 significantly decreased the thermal hyperalgesia and mechanical allodynia and microglia activation as well as inflammatory cytokines in spinal cord. These findings suggest that preconditioning with H_2S may prevent neuropathic pain in rats through microglia deactivation and inflammation inhibition in the spinal cord, in which the PPARγ/p38/JNK pathway is involved. This novel finding may significantly help us to understand the difference between the roles of endogenous H_2S and exogenous H_2S in BCP and present us a new strategy of pain management.

Authors' contributions: LZ and KL contribute equally to this article. LZ and KL designed and carried out the experiments; GW measured the changes of plasma H_2S in patients with BCP and analyzed the relationship between them; TS was responsible for the H_2S inhalation, CG helped to perform the BCP procedure; YM participated in the assessment of thermal hyperalgesia and mechanical allodynia; MB did the Western Blot Analysis; MZ did the statistical analysis.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

This study was supported by Unite special fund of Yunnan provincial science and Technology Department, Kunming Medical University, Yunnan, China (No. 201501UH00628).

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(Received July 15, 2017, Accepted October 5, 2017)