Local Administration of Thiamine Ameliorates Ongoing Pain in a Rat Model of Second-Degree Burn

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The objective of this study was to develop rat model of second-degree burn pain and test analgesic efficacy of local thiamine administration. Automatic temperature-controlled hot plate was set at 85 ± 0.1°C with a filter paper on the top. Rats were thrust on hot plate landing on plantar surface for 4 to 7 and 10 seconds, respectively. Burnt skin was observed. Hematoxylin and eosin staining and Masson staining were used to monitor burn degree. Gait analysis detected change of locomotion. Allodynia and hyperalgesia in the burnt area were evaluated with von Frey test and Hargreaves Test, and ongoing pain was detected with conditional place preference test. Markers for the activity of microglia (Iba1), astrocytes (GFAP), and neurons (c-fos) were detected with immunofluorescence. Finally, thiamine was injected into blisters to observe its effect on burn pain. Blisters on burnt skin, space between dermal and epidermal layers in hematoxylin and eosin staining and burn injury limiting in dermal layer in Masson stain all indicated that burn injury lasting for 7 seconds matched second-degree burn. Behavioral tests revealed allodynia, ongoing pain, and increased expression of c-fos, GFAP, and Iba1, as well as the absence of hyperalgesia in Burn7s. Burn injury reduced distance of second and fourth digits. MK801 could relieve allodynia in Burn7s. Local administration of 1, 2, and 4 mg of thiamine had no effect on the allodynia, but 2 and 4 mg of thiamine also could induce CCP in Burn7s. A rat model of second-degree burn pain was developed and local administration of thiamine provided relief from pain. (J Burn Care Res 2017;XXX:00–00)

In clinical practice, pain is one of the main symptoms of burn injury and an emergency requiring medical assistance, and second-degree burn injury is the most painful condition.[1] When the area of burn was large, pain is unbearable. Therefore, relief of burn-induced pain improves patients' quality of life. A comprehensive understanding of the underlying mechanisms of pain and development of pain medicine require animal models of burn-induced pain simulating clinical conditions.

Although animal models of burn injury were developed, in which the injured area involved animal trunk,[2,3] they were not appropriate for pain research or pain medicine investigation, which required plantar surface injury for almost all pain evaluation system. Therefore, animal models of burn-induced pain need to be redeveloped. Several animal models of burn-related pain were developed recently.[4–9] However, a stable animal model of second-degree burn injury is still rare.

Thiamine has a proven antinociceptive efficacy in acute and chronic pain, and is a potential pain medication with few side effects.[10–12] This conclusion was got from the results that thiamine could relieve hyperalgesia in various animal models. Recent years testing for ongoing pain in any pain animal model was believed to be necessary for the development of pain medicine and prediction of clinical outcome.[13,14] Meanwhile most studies about burn pain also focused on allodynia and hyperalgesia, instead of ongoing pain.[6,15] Therefore, we dedicate to evaluate the effect of thiamine on ongoing pain induced by burn to supply ongoing pain evidences for both thiamine antinociceptive efficacy and burn pain.

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In this study, based on the current animal model of burn-induced pain,[5] we developed an animal model of pain associated with second-degree burn injury and tested the antinociceptive effect of thiamine, especially on ongoing pain.

METHODS

Animals and Modeling

Forty-two adult male or female Sprague-Dawley (SD) rats, weighing 180 to 220 g each, were acquired from the Animal Center of Xuzhou Medical College. Animals were maintained at a temperature of 20 to 23°C, under 49 to 51% moisture and 12 hours:12 hours light/dark cycle, with supply of food and water ad libitum. The animal study was compliant with the rules of the Ethics Committee of the International Association for the study of pain, and all the protocols were approved by the Institutional Animal Care and Use Committee.

The animal model was created using an automated temperature controller (Accublock D1100, Labnet international Inc. Edison, NJ), previously set at 85°C for at least 30 minutes with a filter paper on its hotplate. The left hindpaw of the rats was placed on the surface of the filter paper under slight pressure dorsally to ensure that the plantar surface of the paw closely touched the filter paper. During the process, rats were immobilized with rat holder and anesthetized with isoflurane. These steps lasted for 4, 5, 6, 7, and 10 seconds, representing in different groups of rats, respectively. The temperature was based on other related studies.[4,5]

Histological Observation, Hematoxylin and Eosin Staining, and Masson Staining

Burnt skin was observed 24 hours after burn injury and three rats from each group were killed for hematoxylin and eosin (H&E) staining and Masson staining. Tissue (1 cm^2 from the injured area) was sampled, fixed in 4% paraformaldehyde for 24 hours, and dehydrated in 40% sucrose solution overnight. Slices of 10 µm were obtained using a cryostat (Leica CM1900, Richmond, IL), stained with H-E kit (Beyotime, Nantong, JS, China) or Massson stain kit (Maxim, Fuzhou, FJ) and observed under light microscope.

Paw Withdrawal Mechanical Threshold

Pain behaviors were observed 1 day before burn injury, 2 hours after injury, and 1 to 7 days after injury. Paw withdrawal mechanical threshold was tested with electric von Frey (Ugo Basile, 38450, Milan Italy) with its standard rigid tip. Before testing, rats were placed in a methyl methacrylate box with metal mesh bottom for 30 minutes for acclimation. Using the filament, the injured area was touched and the pressure was increased slowly and evenly. Paw withdrawal was considered as positive. A 15-second time interval was set to avoid adaptation. The final values were derived from the average of the five test values. The test was conducted with groups of six animals each. For the testing time point, paw withdrawal mechanical threshold was performed between 2 and 3 hours after MK801 administration, while MK801 was administrated at 20 minutes before burn injury and 2 hours before testing every day.

Thermal Withdrawal Latency

The test was conducted using a thermal device (Model 390 IITC Life Science, Woodland Hills, CA). Animals were placed on a glass in transparent boxes for 30 minutes to calm down. Radiation was focused on the burn-injured area or the middle of the paw in the control group of animals. The control value was set at about 13 seconds. Two tests involving the same paw were conducted with an 8-minute interval. When the animals withdrew the test paw sharply, the latency time was recorded and recognized as a positive value. Each group comprised six animals and the test was performed.

Thiamine Administration

In the animal models of burn injury, 1, 2, and 4 mg thiamine (Sangon Inc. Shanghai, China), diluted in saline and adjusted to pH 7.0, were injected into the injured area (blister) on days 1, 2, and 3, postoperatively. Rats were immobilized in a holder during the operation and 20 minutes after injection to ensure thiamine absorption by the tissue. Thiamine leakage was controlled by adapting the thin intravenous infusion needle (0.45 mm diameter) and prolonged retention of the needle (20 minutes). The doses were determined based on our previous experiment investigating the effect of pain relief in a formalin pain model, in which local administration of thiamine maximally relieved formalin pain at 2 mg (data not shown). And 1, 2, and 4 mg of thiamine was administered locally in the conditional place preference (CPP) test at 20 minutes before test on conditioning days.

Conditional Place Preference

The customer-designed CPP apparatus included a two-box CPP device. The walls of one conditioning box were white with black vertical stripes and the

walls of the other chamber were white with black dots. The chamber floors were smooth to avoid irritation to the burnt skin on the plantar surface of hindpaw. The CPP procedure spanned six consecutive days. On day 1 postoperatively, rats were placed in the apparatus for 30 minutes with open access to each of the two chambers. On the next day (preconditioning day), the rats subjected to 7 seconds of burn injury were transferred into the device for 15 minutes with free access to the two chambers, and the baseline data were recorded. On days 3, 4, and 5 (conditioning days), saline-administered rats were restricted to a single chamber for 30 minutes in the morning, and rats treated with, MK801 (Selleckchem, Houston, TX) or thiamine (sangong, Nantong, JS, China) were housed together in another chamber for 30 minutes in the afternoon. On the last day (postconditioning day), rats were provided with free access to the two chambers for 15 minutes, and the time spent in either conditioning box was measured. Rats spending less than 20% or more than 80% of their time in either conditioning chamber were excluded from the CPP test. On the basis of these criteria, 17 animals within all 121 animals were removed from CPP analyses. The number of rats in every group would be supplemented to keep at seven to eight if there were rat was excluded out the experiment for above reasons. Placement was balanced within each treatment group to ensure that half of the animals started in one chamber and the other half started in the second chamber. Time spent in each chamber was hand-scored by two observers blinded to experimental conditions. An animal was determined to be "in" a chamber when all four of its paws were situated in that chamber.

Immunofluorescence

The animals were subjected to behavioral testing before they were killed. After deep anesthesia with 10% chloral hydrate solution, the three rats in each group were transcardially perfused with 150 ml saline to replace blood followed by 4% paraformaldehyde to fix the spinal cord. A fixed lumbar spinal cord measuring approximately 5 mm long was immersed in 4% paraformaldehyde solution overnight and subsequently dehydrated in 40% sucrose solution for 3 days. The spinal cord was sliced into 25-μm-thick sections. Free-floating immunofluorescence staining was used to detect the changes in c-fos, GFAP, and Iba1 expression with rabbit anti-c-fos (1:100, Abcam, Cambridge, MA), rabbit anti-GFAP (1:100, Santa Cruz Biotechnology, Santa Cruz, CA), and goat anti-Iba1 (1:400, Abcam, Cambridge, MA) antibodies. Corresponding donkey antirabbit and donkey antigoat secondary antibodies (1:400, Abcam, Cambridge, MA) was used. Images were captured with confocal microscope (Leica SP2, Mannheim, Germany). Eighteen sections from three spinal cords (six slices each) were analyzed. The c-fos number in spinal dorsal horn and the optical density of GFAP and Iba1 were counted.

Locomotion Observation With Gait Analysis

The gaits of rats were collected according to the protocol. In brief, let rats with eosin on its feet to pass a custom-designed passage to leave foot print on white paper.[16] And the distance between second and fourth digits was measured and analyzed.[17] This test was performed at 24 hours after burn injury lasting 7 or 10 seconds. Finally, four to five paired gaits from same rats (n = 6) was analyzed (Figure 6).

Statistical Analysis

All the data were expressed as mean ± standard error (± SEM) and analyzed with SPPSS17.0. The behavioral test data were analyzed using two-way analysis of variance, followed by Bonferroni post hoc test. Other test data were analyzed using one-way analysis of variance followed by Tukey's test. $P < .05$ was considered statistically significant.

RESULTS

Histological Observations

Skin morphology and H&E staining of the 4-second group 24 hours after burn injury showed no differences from that of the control. The 5-second group showed a pink-red burnt skin, and the epidermis separated from the papillary layer of dermis, which was intact. The 6-second group displayed red burnt skin, with a clear border demarcating normal skin, without any macroscopic blisters, while the H&E stain showed papillary layer separating from the other layers. The 7-second group showed blisters with a space between the papillary layer and the other layers of the dermis under H&E staining. The 10-second groups showed a bigger blister than that of the 7-second groups, while the H&E stain displayed features similar to that of the 7-second group (Figure 1).

Burn depth of skin of burn injury lasting 7 seconds was further confirmed with Masson stain. The epidermal layer of skin was stained as red for that its main content was elastic fiber. The epidermal layer became thinner at 48 and 72 hours after burn injury comparing with that of naïve and 24 hours. While dermal layer of skin containing collagenous fibers

Figure 1. Observations of burnt skin at 24 hours after burn injury. The 4-second group showed no differences from sham control. The 5-second group showed pink skin with blurred border surrounded by normal skin. The 6-second group showed red appearance with clear border from normal skin. The 7-second group showed a blister. The 10-second group showed bigger blisters. n = 3. Arrow indicates the burn area.

were stained as blue. Burn injury made the tissue less stained and thus showed the burn depth. At 24 hours after burn injury, burn depth limited at papillary layer in dermal (Figure 3B), while burn depth extended beyond papillary layer (Figure 3C). At 72 hours after burn injury, burn-injured dermal tissue broke away from uninjured one and became a line (Figure 3D). Finally, the 7-second burn was selected to establish the animal model of second-degree burn-induced pain, followed by behavioral testing and immunofluorescence staining.

Second-Degree Burn Injury Induced Mechanical Allodynia, Ongoing Pain But Not Thermal Hyperalgesia

In the von Frey test, the value of control group was maintained stably at 38 ± 2 g, which was different

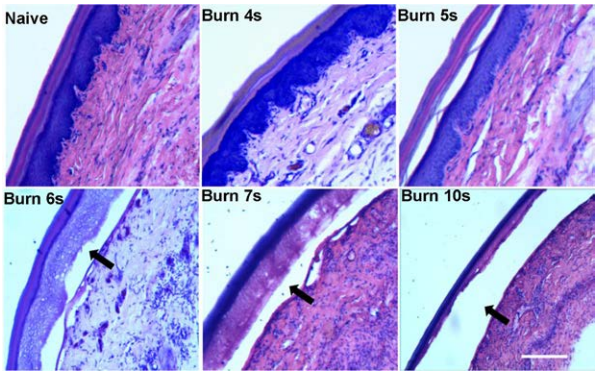

Figure 2. Hematoxylin and eosin staining of burnt skin reveals the degree of burn at different burn times. Compared with the sham group, the burn at 5 seconds showed damaged epidermis; the burn at 6–10 seconds showed dermal injury, with blisters containing spaces in the tissue (arrows). The blisters also confirm that the burn injury was not third degree. Bar = 100 μm. n = 3–4.

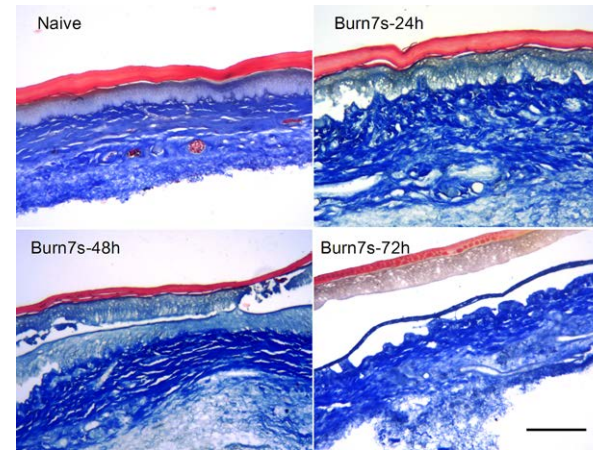

Figure 3. Confirmation of the burn depth in Burn7s with Masson staining. Masson staining was performed at 24, 48, and 72 hours after burn injury in that burn injury usually was under development within 48 or 72 hours. Collagenous fiber was stained as blue and elastic fiber was stained as red. Bar = 100 μm.

from the control data (15 ± 0.2 g) reported in other studies.[18] We believe that this difference was related to the different tip size of the electric von Frey filament. The duration of burn injury varied with the degree of allodynia: the 7-second group showed decreased pain threshold ranging from 2 hours to 3 days, and recovered to control levels on day 7 after the burn injury (Figure 4A). Any difference in withdrawal latency was not detected in the controls during the Hargreaves test (Figure 4B). To find out whether ongoing pain existed in this animal model, intraperitoneal administration of MK801 on 1, 2, and 3 days after burn injury induced CPP in animal models of second-degree burn pain (Figure 4C). And intraperitoneal administration of MK801 also could relieve the mechanical allodynia induced by burn injury lasting for 7 seconds (Figure 4D).

Immunofluorescence

Immunofluorescence testing on day 3 after burn injury lasting for 7 seconds (Figure 5) showed increase in c-fos, Iba1, and GFAP expression in the spinal dorsal horn, containing the three main cell categories (neurons, microglia, and astrocytes) involved in pain.[18]

Locomotion After Burn Injury

To observe the influence of burn injury for the locomotion, gait analysis was performed. The results showed that the distance between second and fourth digits reduced in burn-injured foot compared with contralateral foot of the same rat, and difference values (*D* values) of second and fourth digits distances

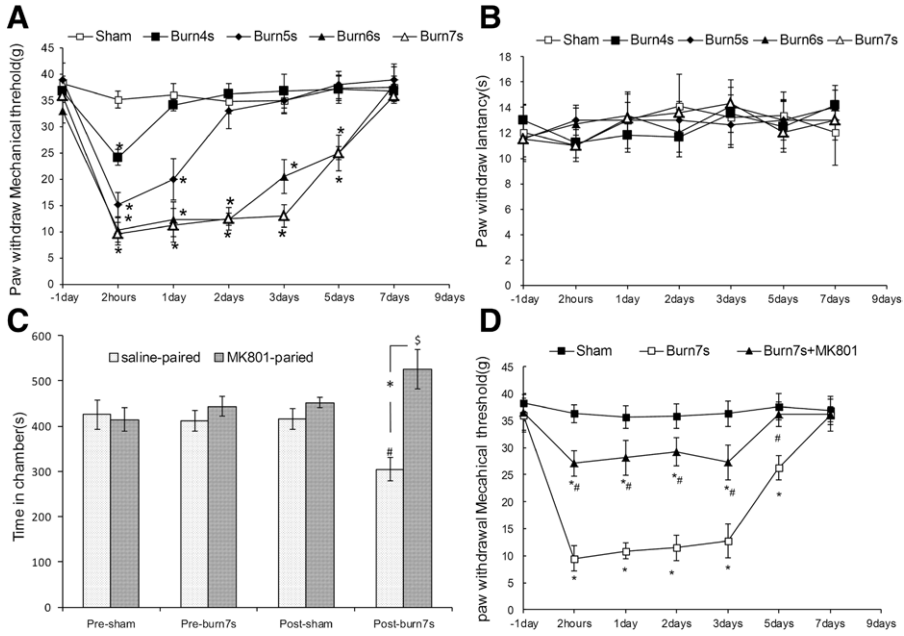

Figure 4. Von Frey test and Hargreaves test were conducted to evaluate burn injury lasting 4–7 seconds and conditioned place preference (CPP) test was performed in Burn7s. A. von Frey test showed that the paw withdrawal threshold decreased with increase in burn time. Compared with the sham group, the burn injury lasting for 5 seconds (Burn5s) showed decreased paw withdrawal mechanical threshold at 2 hours to 1 day after the burn injury, and burn injury lasting for 7 seconds (Burn7s) showed a stable decreased in paw withdrawal mechanical threshold at 2 hours after burn injury until 3 days later and returned to sham group level within the next 4 days, but still showed significant difference from the sham group at 5 days postinjury. Compared with the Burn5s group, the Burn7s also showed significant differences at 2 hours, 1 day, 2 days, 3 days, and 5 days after injury (*$P < .05$, compared with the sham group, #$P < .05$, compared with the Burn5s group). B. Hargreaves test indicated no differences among groups. C. Intraperitoneal injection of MK801 (0.03 mg/kg) induced conditioned place preference in Burn7s injury. Time spent in the saline-paired chamber during preconditioning was similar. In sham rats (n = 8), there was no change in preference during postconditioning (post) when compared with preconditioning baselines (pre). In Burn7s rats (n = 8), MK801 prolonged the time spent in the MK801-paired chamber when compared with preconditioning baseline. These results taken together indicate that MK801 induced CPP in burn-injured rats, thereby relieving ongoing pain in rats with second-degree burn injury. #$P < .05$, significant difference from "preburn7s saline-paired." \$$P < .05$, significant difference from "preburn7s MK801-paired." *Significant difference between indicated groups. D. The effect of MK801 on allodynia induced by second-degree burn injury. MK801 was intraperitoneally administrated at 20 minutes before burn injury and 2 hours before testing every day. *$P < .05$, compared with sham. #$P < .05$, compared with Burn7s group.

of injured and noninjured foot were significantly different between Burn7s or Burn10s group and naïve (Figure 6).

Thiamine Treatment

Local administration of 1, 2, or 4 mg thiamine had no effect on mechanical allodynia (data not shown for administration of 1 and 2 mg thiamine; Figure 7A). However, intradermal injection of 2, 4 mg thiamine induced CPP in rats with second-degree burn pain (Figure 7C, D).

DISCUSSION

Until now, most animal models of pain associated with burn injury involved whole layers of skin matching third-degree burn clinically. The second-degree burn injury is a challenge because conditions such as temperature and time need to be controlled precisely. Compared with other types of burn induction such as immersion in hot water,[15] injury with chemicals[19,20] or radiant heat,[7] the hot plate method is the most popular due to its simplicity and controlled process.[5] In this study, we adapted the hot plate method to develop a stable second-degree pain model and investigate the antinociceptive effect of thiamine.

To establish the animal model of pain associated with burn injury, a temperature of 85°C lasting for 15 seconds was used to induce third-degree injury.[5] Although in theory, all possible combinations of temperature and time were used to induce burn injury, 85°C was still adopted in our animal model of second-degree burn injury to maintain continuity with other burn injury-induced pain models.

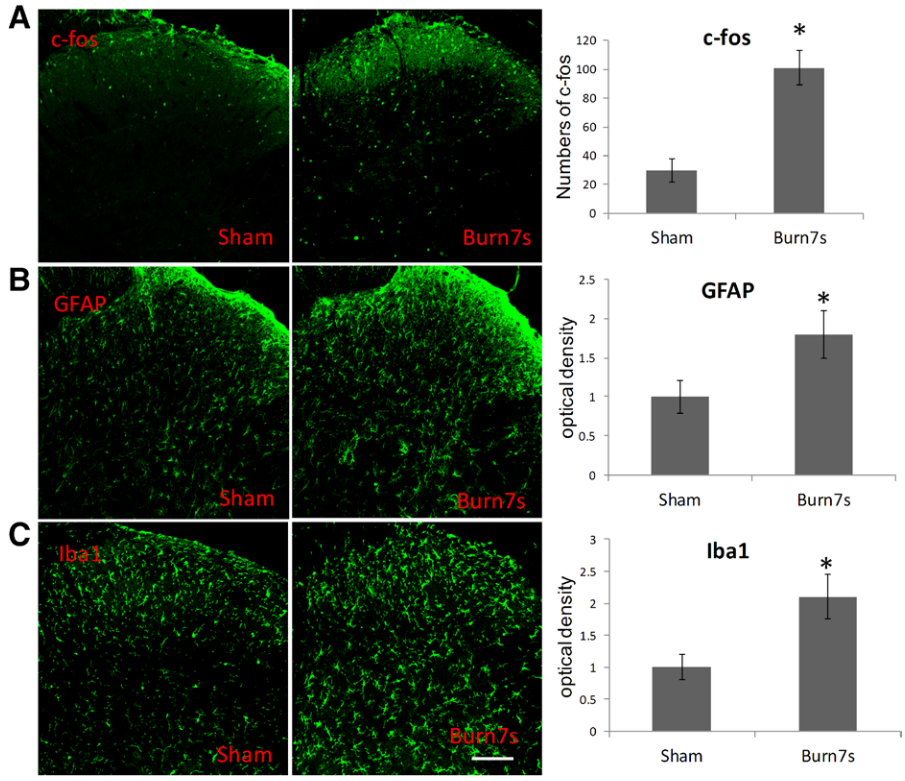

Figure 5. Increased expression of c-fos, GFAP, and Iba1 in the spinal cord 3 days after burn injury in Burn7s. A. c-fos expression in sham group and Burn7s group. B. GFAP expression in sham group and Burn7s group and Iba1 expression in sham group and Burn7s group.*$P < .05$ compared with the sham group. Bar = 100 μm.

Two methods were adapted to improve the stability of the burn pain. First, an automated temperature controller was introduced to accurately control the temperatures to 0.1°C.[5] Increased accuracy in temperature control ensured stability of the burn. Second, a filter paper was inserted between the hot plate and the paw. Initially, we created the second-degree burn exactly according to the protocol in which the plantar skin was directly connected to the hot plate.[5] However, the degree of injury was not uniform because part of the plantar skin touched the hot plate first and was rapidly injured, We, therefore, used a filter paper on the top of the hot plate to delay the heat reaching the skin and ensured that the degree of burn injury was uniform, as demonstrated by our H&E staining protocol (Figure 2). Furthermore,

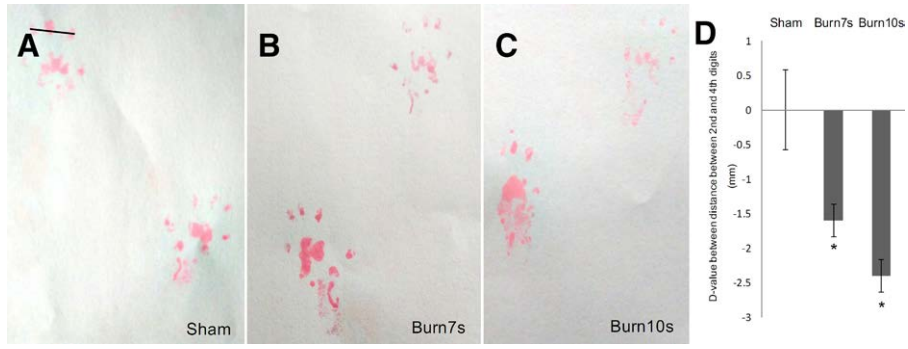

Figure 6. The effect of burn injury on locomotion. A. Foot prints was collected from sham rats. The line indicates the distance between second and fourth digits. B. Foot prints were collected from Burn7s rats at 24 hours after burn injury. C. Foot prints were collected from Burn10s rats at 24 hours after burn injury. D. The different values (*D* value) between distance of second and fourth digits of injured foot and uninjured foot were statistically analyzed among groups. *$P < .05$, compared with sham.

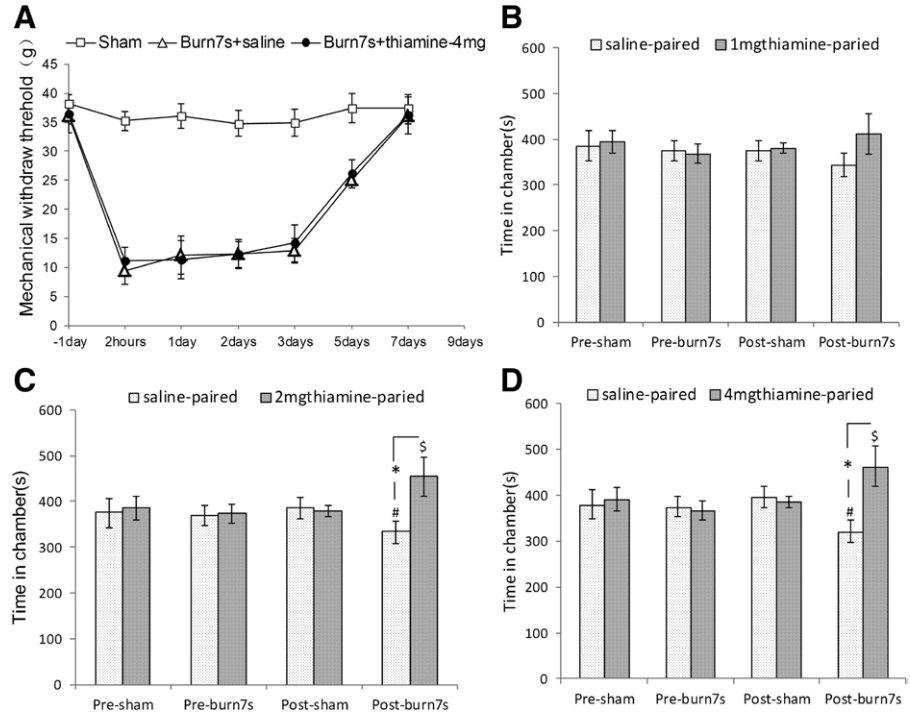

Figure 7. Local administration of thiamine failed to relieve mechanical allodynia, but induced conditional place preference in Burn7s. A. At 1, 2, and 3 days after burn injury, thiamine (4 mg) was locally injected into the blister. B. In both sham and Burn7s rats (n = 6), no changes were observed during postconditioning (post) when compared with preconditioning baselines (pre). 1 mg thiamine was administrated during conditioning days. C. In sham rats (n = 8), no changes were observed during postconditioning (post) when compared with preconditioning baselines (pre). In Burn7s rats (n = 8), thiamine increased the time spent in the thiamine-paired chamber when compared with preconditioning baseline. 2 mg thiamine was administrated during conditioning days. D. In sham rats (n = 8), no changes were observed during postconditioning (post) when compared with preconditioning baselines (pre). In Burn7s rats (n = 8), thiamine increased the time spent in the thiamine-paired chamber when compared with preconditioning baseline. 4 mg thiamine was administrated during conditioning days. #$P < .05$, significantly different from "preburn7s saline-paired"; \$$P < .05$, significantly different from "preburn7s thiamine-paired";*$P < .05$, significant difference between indicated groups.

appropriate immobilization of the rat, for instance, placing the animal in a holder, also ensured the stability of the model.

Burn injury was classified into three degrees and six classes according to H&E stain.[21] Without H&E stain confirming the degree of burn, the burn-induced pain model could not be related with clinical conditions because pain varies with the degree of injury.[9,22] The second-degree burn was the most painful, and the third-degree burn resulting in ongoing pain was milder,[21] although pain might be induced in the area surrounding the injury.[1,5,23] Here, we focused on pain associated with second-degree burn injury. H&E stain indicated that burn injury reached the epidermal papillary layer, as well as the shallow layer of dermis in second-degree burn,[21] whereas Masson stain is proved to be a better method to reveal the depth of burn.[24] In this study, a temperature of 85°C for 7 seconds induced a second-degree burn in which the epidermal papillary layer separated from the dermis and the cells in the papillary layer were damaged and unstainable (Figures 2 and 3). Thus, H&E and Masson staining results revealed that the injured dermal layer was less stained compared with the intact dermal layer (Figure 3). Combined with the existence of blisters, which is the characteristic of non-full-thickness burn injury,[25] the evidence of Masson stain in burn injury lasting from 7 to 10 seconds revealed that burn depth limited to dermal layer, and, therefore, this animal model could be classified as a second-degree burn animal model. Although Masson stain at 48 hours after burn revealed that the burn depth increased during this period, it still was limited to the dermal layer (Figure 3C). Behavioral tests showed that a burn time of 7 seconds induced stable allodynia at 2 hours to 3 days after burn (Figure 4A). Furthermore, the expression of c-fos, Iba1, and GFAP was increased in the 7-second burn apparently, suggesting that the three markers reflected pain, at least

partially (Figure 5). The foregoing evidences suggested that the 85°C burn lasting for 7 seconds was the most preferable temperature to induce second-degree burn injury in a rat model.

It is natural that burn injury may influence locomotion through inducing wound or pain. The gait analysis in this study revealed that the burn injury indeed affected the locomotion, which was indicated by the reduced the distance of second and fourth digits (Figure 6). the gait analysis has been regarded as a candidate indicator of pain in recent years, which is especially suitable for the pain type influencing locomotion, such as osteoarthritis-induced pain and tendon injury-induced pain.[26,27] The gait analysis may also be suitable for burn-induced pain because this type of pain is similar to that induced by an intraplantar injection of carrageenan.[28]

The von Frey filament and Hargreaves tests revealed mechanical allodynia; however, thermal hyperalgesia did not exist in burn injuries lasting for 4 to 7 seconds. Stable allodynia, which lasted from 2 hours to 3 days after the operation, was helpful in burn-induced pain research. Moreover, the absence of thermal hyperalgesia in burn injury, concurrent with other researches,[15] might be a characteristic of burn pain, even in a third-degree burn injury.[5] Although this characteristic would not greatly interfere with demonstrating the mechanism of burn pain and developing burn pain-relieving medicine or treatment, it indicated that these burn models were not suitable for hyperalgesia-related studies or medical researches. Alternatively, it would be an interesting research topic form a different perspective why thermal hyperalgesia was absent in the burn animal model. In this study, a local administration of thiamine had no effect on allodynia associated with burn injury (Figure 7A), consistent with a previous study in which thiamine failed to alleviate neuropathic pain.[12]

Ongoing pain is the main symptom of burn-induced pain clinically.[1] It was widely accepted that ongoing pain, aside from allodynia and hyperalgesia, should be investigated in animal models.[14,29] Initially, we expected to observe signs of ongoing pain, such as paw withdrawal or licking, which were regarded as signs of ongoing pain in formalin-induced pain models.[30] However, surprisingly, neither paw withdrawal nor licking was observed, consistent with other studies.[5,15] We believe that paw withdrawal and licking are not signs of ongoing pain but only represented sudden pain observed in the hot plate test, and intermittent pain in formalin-induced pain models. Fortunately, CPP was developed to detect the existence of ongoing pain[14,29] and was the only accepted method detecting ongoing pain at present. The CPP paradigm is based on traditional learning principles and involves the pairing of drug state and environment which have distinctive stimuli (ie, place). The whole CPP process for detecting pain was regularly divided into three phases-preconditioning phase, conditioning phase, and postconditioning phase. During the conditioning phase, the relieving pain effect of drugs (ie, MK801) was supposed to paired with the chamber in animal model and as a result animals was inclined to stay in drug paired chamber. So during the next postconditioning test, even when the drug was inhaled, animals still would exhibit prolonged time in paired chamber. While in drug development, when a drug prolonged the time in paired chamber, it was consider to be effective for relieving pain.[31,32] To investigate ongoing pain in this second-degree burn-induced pain model, MK801 was administrated intraperitoneally. The result showed that 0.03 mg/kg MK801[33] induced CPP at 1 to 3 days postoperatively in this animal model (Figure 4C) and suggested the existing of ongoing pain in this burn animal model. Local administration of thiamine (2 and 4 mg) also induced CPP (Figure 7C, D). This result suggested that thiamine could be relieving ongoing pain of burn injury and might be used clinically to relieve second-degree burn pain. And local administration of thiamine may be an effective way. It also was reported that thiamine could benefit the wound healing through enhancing the collagen synthesis.[34] Combining antinociceptive effect and wound healing effect decided thiamine was as an idle medicine for burn injury. By the way, we failed to observe the dose-dependent effect of thiamine on ongoing pain with CPP assay partly because CPP assay was not precise enough to distinguish the effect of different dosage of thiamine on ongoing pain. As a support, CPP assay was seldom used to detect dose-dependent effect of other medicines too.[14,29,33,35–37] This might be limitation for present CPP assay which should be modified in the future.

Until now, there is no exquisite theory to explain the mechanism underlying the antinociceptive effect of thiamine. It is reported that thiamine could improve the effect of wound healing through ensuring the energy supply for cells synthesizing collagen by facilitating ATP production.[34] Moreover several researches observed that pain was reduced when wound healing was improved in posthemorrhoidectomy[38] and episiotomy.[39] Therefore, the possibility exists that thiamine improves wound healing, which, in turn, leads to mitigation of burn pain. More studies are needed to demonstrate the mechanism of antinociceptive effect of thiamine.

In summary, we developed an animal model of pain induced by stable second-degree burn injury and local administration of thiamine attenuated the pain, suggesting potential clinical application.

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