Research Article

MOLECULAR PAIN

Dimethyl fumarate attenuates pain behaviors in osteoarthritis rats *via* induction of Nrf2-mediated mitochondrial biogenesis

Molecular Pain
Volume 18: 1–12

DOI: 10.1177/17448069221124920
journals.sagepub.com/home/mpx
SAGE

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Abstract

Osteoarthritis (OA), a chronic degenerative disease, leads to pain and loss of function. Existing treatments for OA pain have limited efficacy and show significant side effects. Dimethyl fumarate, a robust nuclear factor erythroid 2-related factor 2 (Nrf2) activator, could alleviate pain behaviors in chronic pain. This study aims to investigate the role of dimethyl fumarate in a rat model of OA induced by monosodium iodoacetate (MIA) and its underlying mechanisms. We used von Frey filaments to assess the mechanical allodynia. Weight-bearing apparatus was employed to assess the hindlimb weight distribution. Western blot was employed to investigate the protein expressions of mitochondrial biogenesis markers. RT-qPCR was employed to examine the copy number of mitochondrial DNA (mtDNA). In our study, dimethyl fumarate upregulated mechanical paw withdrawal threshold (MPWT) (MIA + Vehicle, 1.6 ± 0.13 *g* [mean ± SEM]; MIA + Dimethyl fumarate (DMF) 300 mg/kg, 10.5 ± 0.96 *g*; $p < 0.0001$). Hindlimb weight distribution was also upregulated by dimethyl fumarate (MIA + Vehicle, 38.17 ± 0.72 *g*; MIA + DMF 300 mg/kg, 43.59 ± 1.01 *g*; $p < 0.01$). Besides, activation of Nrf2 remarkably upregulated the protein levels of PGC-1α (MIA + Vehicle, 0.69 ± 0.07; MIA + DMF 300 mg/kg, 1.08 ± 0.09; $p = 0.0037$), NRF1 (MIA + Vehicle, 0.69 ± 0.04; MIA + DMF 300 mg/kg, 1.00 ± 0.11; $p = 0.0114$), TFAM (MIA + Vehicle, 0.62 ± 0.11; MIA + DMF 300 mg/kg, 1.02 ± 0.12; $p = 0.0147$), and the copy number of mtDNA (MIA + Vehicle, 0.52 ± 0.05; MIA + DMF 300 mg/kg, 3.81 ± 0.21; $p < 0.0001$). Taken together, these results show that dimethyl fumarate alleviated pain-related behaviors in a rat model of OA through activation of Nrf2-induced mitochondrial biogenesis.

Keywords

Dimethyl fumarate, osteoarthritis pain, Nrf2, mitochondrial biogenesis

Introduction

Osteoarthritis (OA), one of the most disabling musculoskeletal conditions, is a huge burden to the social economy and affects the patient's quality of life.[1] Pain is a remarkable symptom of OA.[2] The type of OA pain is still up for debate. Nerve damage, inflammatory, and damaged joint tissues might be the causes of OA pain.[3–6] According to European League Against Rheumatism (EULAR) guidelines, pharmacologic treatment of OA pain relies primarily on NSAIDs and opioids.[2] NSAIDs are insufficient to alleviate pain in OA, and opioids can produce significant adverse effects including nausea, dizziness, somnolence, respiratory depression, and vomiting. Therefore, an attractive therapeutic target needs to be further studied.

Reactive oxygen species (ROS) are implicated in chronic pain development through several mechanisms, including oxidative stress and mitochondrial biogenesis impairment.[7–11] Our previous study has shown that ROS scavengers could alleviate cancer-induced bone pain.[8]

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Mitochondria participate in many physiological processes, such as energy production, calcium homeostasis, and cell death. Mitochondrial biogenesis is defined as the process of generating mitochondrial DNA (mtDNA), mitochondrial proteins, and new mitochondria.[12] Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) regulates this process by promoting the expression of nuclear respiratory factors 1 and 2 (NRF1, NRF2) and mitochondrial transcription factor A (TFAM).[13] Restoring mitochondrial biogenesis attenuated pain behaviors in a rat model of OA, paclitaxel-induced neuropathic pain, and SNI-induced neuropathic pain.[14–16] Therefore, it is plausible that restoring mitochondrial biogenesis and redox balance might attenuate pain behaviors.

Nrf2 is involved in antioxidant defense as the main regulator.[17,18] Although present studies have shown that Nrf2 activators have analgesic effects, the potential mechanisms remain elusive.[19] For this reason, we have focused on Nrf2. Under normal circumstances, Nrf2 binds to Kelch-like ECH associated-protein 1 (Keap1) to form a complex, and then this complex is ubiquitinated for degradation. When oxidative stress occurs, Nrf2 is dissociated from Keap1 and translocated to the nucleus. When Nrf2 enters the nucleus, it binds to the antioxidant response elements to initiate the transcription of antioxidant-related genes.[20] Recent evidence has indicated that Nrf2 promotes mitochondrial biogenesis via regulating PGC-1α.[21] Moreover, our recent study has shown that RTA-408, an Nrf2 inducer, alleviated neuropathic pain via restoring mitochondrial biogenesis.[22] Thus, Nrf2 is a promising therapeutic target to restore mitochondrial biogenesis.

Dimethyl fumarate (DMF), a potent Nrf2 activator, is favored by FDA of the United States to treat relapsing-remitting multiple sclerosis.[23] It has been shown that DMF plays a vital role in neurological disease that could activate Nrf2 and produced a neuroprotection effect.[24,25] Besides, DMFalleviated migraine induced by nitroglycerin in mice.[26] Moreover, In a mouse model of spared nerve injury, DMFcould markedly alleviate pain behaviors in neuropathic pain.[27] Additionally, several lines of evidence have demonstrated that in other diseases, DMFrestored mitochondrial biogenesis by activating Nrf2.[28,29] Thus, our present study investigated that dimethyl fumarate, attenuated pain behaviors in OA rats through restoring mitochondrial biogenesis.

Materials and methods

Animals

SPF-rated adult male SD rats starting weight of 200–220 *g* supplied from the Experimental Animal Center of Tongji Hospital (Wuhan, China) were applied. These animals were kept in a SPF-rated facility that have 12 h of light and have free access to food and water. All animals' experiments were approved by the ethics committee of the Animal Care and Use Committee of Tongji hospital of HuaZhong University of Science and Technology. In the present study, animals were randomly grouped by randomization tools (https://www.random.org/lists/), and behavior tests were sequentially performed on each group of animals.

Establishment of the MIA-induced OA model and behavioral tests

A solution of monosodium iodoacetate (MIA; Sigma-Aldrich, USA) was prepared with saline solution. Mother liquor concentration was 20 mg/mL and it was stored at −80°C. Rats were first anesthetized with 2.5% sevoflurane and intraarticularly administrated MIA (1 mg/50 μL) on day 0 to induce OA.[14] The control group was intraarticularly injected with the same volume of sterile saline solution (0.9%). After injection, animals were placed on a pre-prepared far-infrared warming pad in order to keep their body temperature between 35°C and 37°C until they woke up and we put them back into their cage. These animals didn't receive postoperative analgesia after surgery which was avoided confounding the results of subsequent experiments.

Pain-related behavior tests including mechanical paw withdrawal threshold (MPWT) and weight-bearing asymmetry were experienced. As described earlier,[30] we used von Frey filaments (Stoelting, Wood Dale, IL, USA) to examine MPWT. Firstly, rats were acclimated in plexiglass boxes for 30 min for 3 days in a row. Next, seven von Frey filaments from 1.4 *g* to 15 *g*, were employed to the left hind paw's mid plantar area for 6s. The rat's positive responses to hind paw stimulation consist of one or more elements as follows: (1) licking; (2) shaking; (3) sudden claw retraction. When one of the rat's positive responses was observed more than three times, the reaction was considered positive. Otherwise, a stronger von Frey filament was applied until a positive reaction was observed. Based on our previous studies described,[14] weight-bearing apparatus (TECHMAN, Chengdu, China) was used to assess the hindlimb weight distribution. Pain-related behaviors were experienced between 8:00 $_{AM}$ and 4:00 $_{PM}$. All animals' experiments were conducted blind to group assignment.

Drug administration

Dimethyl fumarate (DMF; Sigma-Aldrich, USA), a potent Nrf2 activator, was suspended in carboxymethylcellulose sodium (Solarbio, China) and administrated by oral gavage. The doses (30, 100, 300 mg/kg) of DMF used in this study were based on a previous study.[27] An Nrf2 inhibitor, trigonelline hydrochloride (Sigma-Aldrich, USA), was dissolved with 0.9% saline solution and administrated by intraperitoneal injection. The experimental design protocol is described in Figure 1. To clarify if consecutive

administration of dimethyl fumarate, an Nrf2 activator, can attenuate established OA pain, dimethyl fumarate (30, 100, 300 mg/kg) was orally administered once a day from day 14 for 5 days in a row. All behavioral tests were experienced 2 hours after dimethyl fumarate administration every day. To clarify whether dimethyl fumarate, can abolish the development of OA pain in the early stage of OA, starting from day 0, rats were orally administrated dimethyl fumarate (300 mg/kg) once a day for 7 days in a row. All behavioral tests were experienced on day 0 before the rat model was built, and on day 3, day 7, day 14, and day 21. To clarify if an Nrf2 inhibitor, trigonelline, can abolish the effect that dimethyl fumarate attenuate OA pain, 20 mg/kg trigonelline was intraperitoneal injection 30min before dimethyl fumarate. All behavioral tests were experienced 30 min before inhibitor administration and 2 h after dimethyl fumarate administration.

Western blotting

When the rats were first anesthetized with 2.5% sevoflurane, the lumbar section of spinal cords was quickly removed, and placed in ice-cold mixture of radioimmunoprecipitation assay lysis buffer containing phosphatase inhibitor and phenylmethylsulfonyl fluoride (Boster; Wuhan, Hubei, China). The concentration of supernatants was examined by the Protein Assay Kit of Bicinchoninic Acid (BCA) (Boster). Then, the proteins were heated at 100°C for 15min with loading buffer and stored at −80°C. The SDS PAGE (10%) was used to separate the samples, 30 μg protein, and then the samples

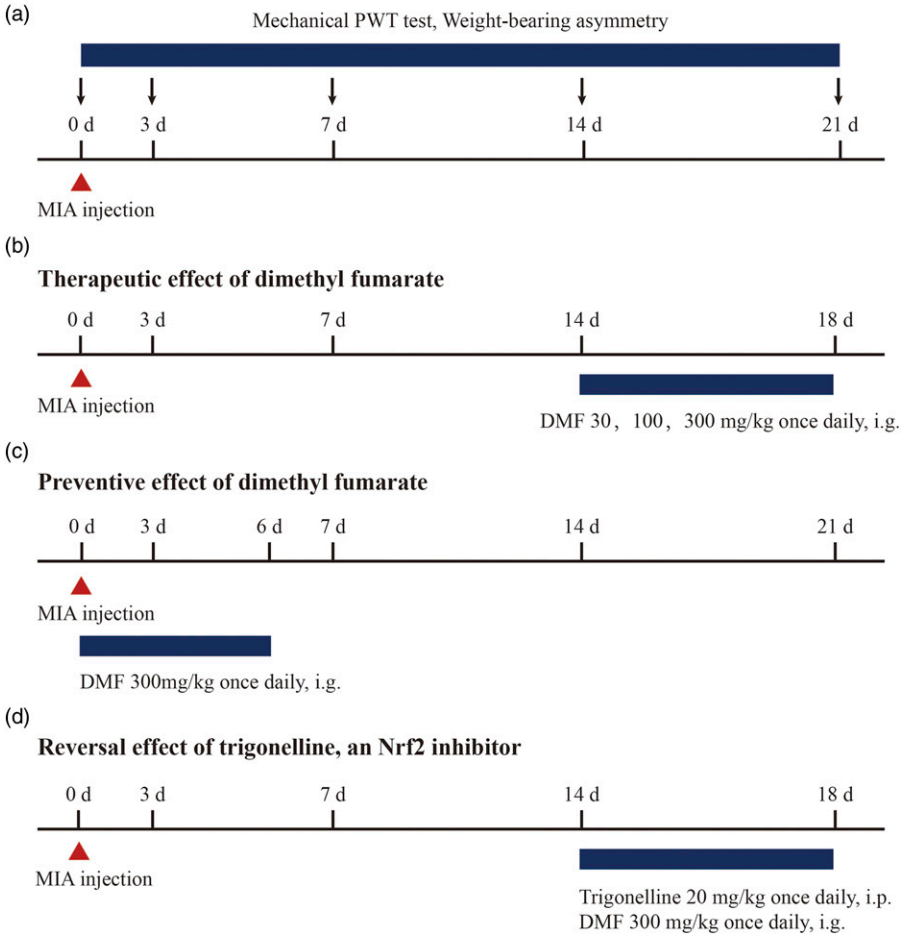

Figure 1. Schematic diagram of the experimental design. (A) Changes in pain-related behaviors after MIA injection in rats. (B) To clarify if consecutive administration of dimethyl fumarate, an Nrf2 activator, can attenuate established OA pain, dimethyl fumarate (30, 100, 300 mg/kg) was orally administered once a day from day 14 for 5 days in a row. All behavioral tests were experienced 2 hours after dimethyl fumarate administration every day. (C) To clarify whether dimethyl fumarate, can abolish the development of OA pain in the early stage of OA, starting from day 0, rats were orally administrated dimethyl fumarate (300 mg/kg) once a day for 7 days in a row. All behavioral tests were experienced on day 0 before the rat model was built, and on day 3, day 7, day 14, and day 21. (D) To clarify if an Nrf2 inhibitor, trigonelline, can abolish the effect that dimethyl fumarate attenuate OA pain, 20 mg/kg trigonelline was intraperitoneal injection 30min before dimethyl fumarate. All behavioral tests were experienced 30 min before inhibitor administration and 2 h after dimethyl fumarate administration.

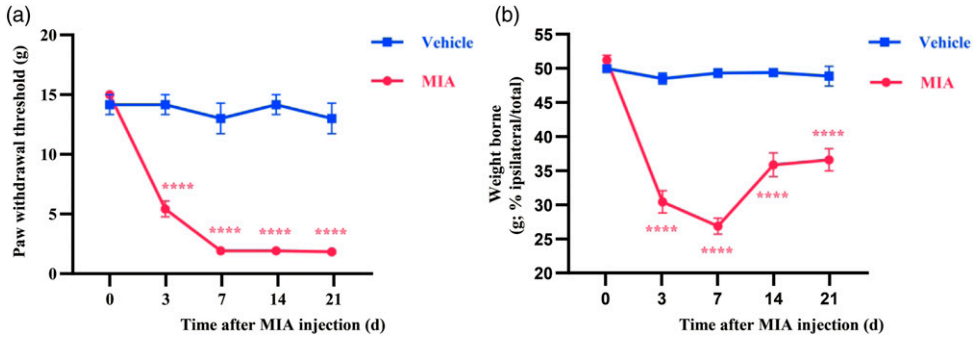

Figure 2. Intraarticular injection of MIA induced pain-related behaviors. (A) Compared with vehicle-treated rats, the ipsilateral MPWT of MIA-treated rats was significantly decreased beginning at day 3 and persisting till to day 21 at least (****$p < 0.0001$ vs. Vehicle group, $n = 6$ rats/group). (B) The Weight-bearing asymmetry was significantly decreased beginning at day 3 and persisting till to day 21 at least (****$p < 0.0001$ vs. Vehicle group, $n = 6$ rats/group).

were transferred to 0.45 μm polyvinylidene fluoride membranes (PVDF; Millipore, USA). Next, the membranes were placed in 5% nonfat dry milk with 0.1% Tween-20 for 90 min at 24°C to block nonspecific binding sites. The membranes were placed in the following antibodies solution overnight at 4°C: rabbit anti β-actin antibody (1:200,000; rabbit monoclonal IgG; AC026; ABclonal), anti Nrf2 antibody (1:1000; rabbit polyclonal; AF7006; Affinity), anti PGC-1α antibody (1:3000; rabbit polyclonal; ab54481; Abcam), anti NRF1 antibody (1:4000; rabbit monoclonal; ab175932; Abcam), anti TFAM antibody (1:2000; rabbit monoclonal; ab252432; Abcam). The membranes were next washed for 15 min and placed in HRP-conjugated secondary antibody solution (1: 5000; goat polyclonal; Aspen) for 1 h at 24°C. Lastly, the protein on membranes was visualized using the ECL Enhanced KIT (RM00021; ABclonal) and ChemiDoc XRS_+ imaging system (Bio-Rad, CA, USA) were used to expose. Image Lab software system (Bio-Rad laboratories) were used to analyze the intensity of protein expression and the protein expressions of mitochondrial biogenesis markers were normalized to the protein expression of β-actin.

Real-time polymerase chain reaction

According to our past studies, we used the DNA extraction kit to extract mtDNA from the lumbar section of the spinal cord.[14,16] qPCR was performed with SYBR Premix kit (EQ001, Wuhan, China). The level of mitochondrial gene ND1 (mtND1) was measured relative to the level of β-actin by the ΔΔCt method.

Statistics

All data are represented as means ± SEM. Prism version 8.0 (GraphPad) was used to analyze all data. The data from western blot and qPCR were tested by one-way ANOVA, followed by Bonferroni *post hoc* test. The behavioral data were tested by two-way ANOVA, followed by Bonferroni *post hoc* test. Statistical significance was defined as $p < 0.05$.

Results

Intraarticular injection of MIA induced pain-related behaviors

Firstly, we observed the mechanical allodynia between Vehicle rats and OA rats induced by MIA intraarticular injection. As shown in Figure 2(a), The ipsilateral MPWT of MIA-treated rats was significantly decreased beginning at day 3 and persisting till to day 21 at least compared with vehicle-treated rats (****$p < 0.0001$ vs. Vehicle group, $n = 6$ rats/group). Conversely, mechanical allodynia was not observed in vehicle group during the study period. As shown in Figure 2(b), Weight-bearing asymmetry was remarkably decreased beginning at day 3 and persisting till to day 21 at least (****$p < 0.0001$ vs. Vehicle group, $n = 6$ rats/group). Based on these results, intraarticular injection of MIA (1 mg/rat) successfully induces pain-related behaviors in rats.

Spinal mitochondrial biogenesis impairment in OA rats

To clarify if mitochondrial biogenesis changes in OA rats, the protein expressions of mitochondrial biogenesis markers were examined in the lumbar section of the spinal cord. In comparison with Vehicle-treated rats, the protein expressions of mitochondrial biogenesis markers were remarkably downregulated in OA rats (Figure 3(a) to (c), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. Vehicle group, $n = 6$ rats/group). Based on these results, mitochondrial biogenesis was markedly repaired in the rat model of OA.

Expression of Nrf2 in the spinal cord of OA rats

The protein expression of Nrf2 was measured by Western blotting. The protein expression of Nrf2 was remarkably decreased in OA rats from day 3 following MIA injection to day 21 (Figure 4(a), **$p < 0.01$, ***$p < 0.001$ vs. Vehicle

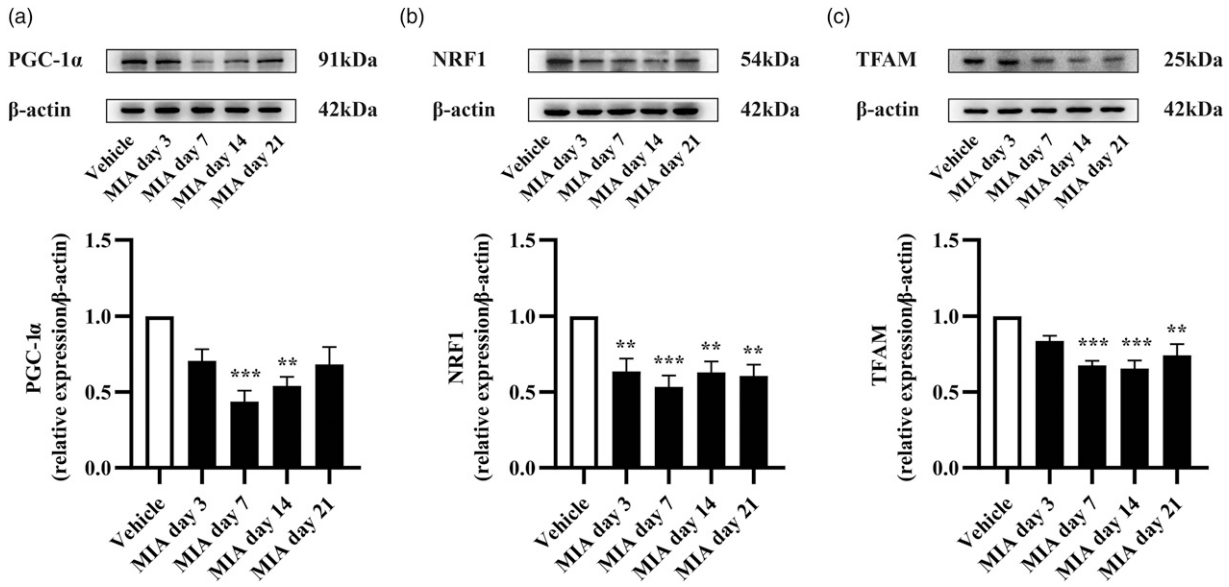

Figure 3. Spinal mitochondrial biogenesis impairment in OA rats. (A) After MIA administration, PGC-1α was remarkably downregulated in OA rats from day 7 to day 14 (**$p < 0.01$, ***$p < 0.001$ vs. Vehicle group, $n = 6$ rats/group). (B) After MIA administration, NRF1 was remarkably downregulated in OA rats from day 3 to day 21 (**$p < 0.01$, ***$p < 0.001$ vs. Vehicle group, $n = 6$ rats/group). (C) TFAM was remarkably downregulated in OA rats from day 7 to day 21 after MIA injection (** $p < 0.01$, ***$p < 0.001$ vs. Vehicle group, $n = 6$ rats/group).

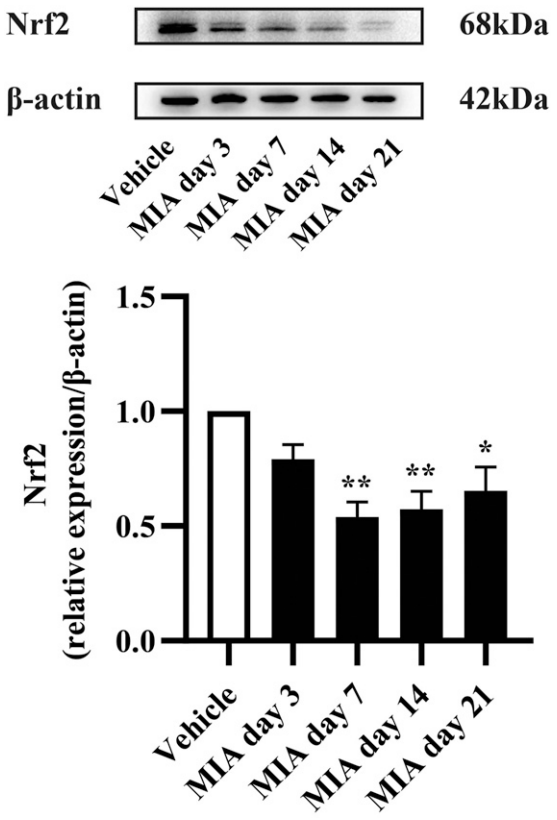

Figure 4. Expression of Nrf2 in the spinal cord of OA rats. (A) The protein level of Nrf2 was remarkably decreased in OA rats from day 3 after MIA injection to day 21 (**$p < 0.01$, ***$p < 0.001$ vs. Vehicle group, $n = 6$ rats/group).

group, $n = 6$ rats/group). This result indicates that the antioxidant defense system was impaired in OA rats.

Analgesic effect of Nrf2 activator attenuated pain-related behaviors in a rat model OA

To clarify if dimethyl fumarate could alleviate pain behaviors in OA rats, dimethyl fumarate (30, 100, 300 mg/kg) was orally administrated once a day from day 14 for 5 days in a row. As illustrated in Figure 5(a), mechanical allodynia was remarkably upregulated by repetitive oral administration of dimethyl fumarate (300 mg/kg) in MIA + DMF 300 mg/kg group (*$p < 0.05$, ****$p < 0.0001$ vs. MIA + Vehicle group, $^{\#\#\#\#}p < 0.0001$ vs. Vehicle + Vehicle group, $n = 6$ rats/group). Weight-bearing asymmetry was markedly reversed at day 18 (Figure 5(b) * $p < 0.05$ vs. MIA + Vehicle group, $^{\#\#\#\#}p < 0.0001$ vs. Vehicle + Vehicle group, $n = 6$ rats/group). Compared with vehicle-treated rats, mechanical allodynia and weight-bearing asymmetry had no change in the 30 mg/kg and 100 mg/kg groups. These data indicate that pain-related behaviors were remarkably alleviated by repetitive oral administration of dimethyl fumarate (300 mg/kg) in a rat model of OA.

To clarify if dimethyl fumarate could abolish pain behaviors in the early stage of OA pain development, 300 mg/kg dimethyl fumarate was orally administrated once a day for seven consecutive days from day 0. In comparison with vehicle group, the MPWT was markedly upregulated from day 3 for 5 days in a row in DMF-treated OA rats (Figure 5(c) and (d), ****$p < 0.0001$ vs. MIA + Vehicle group, $n = 6$ rats/

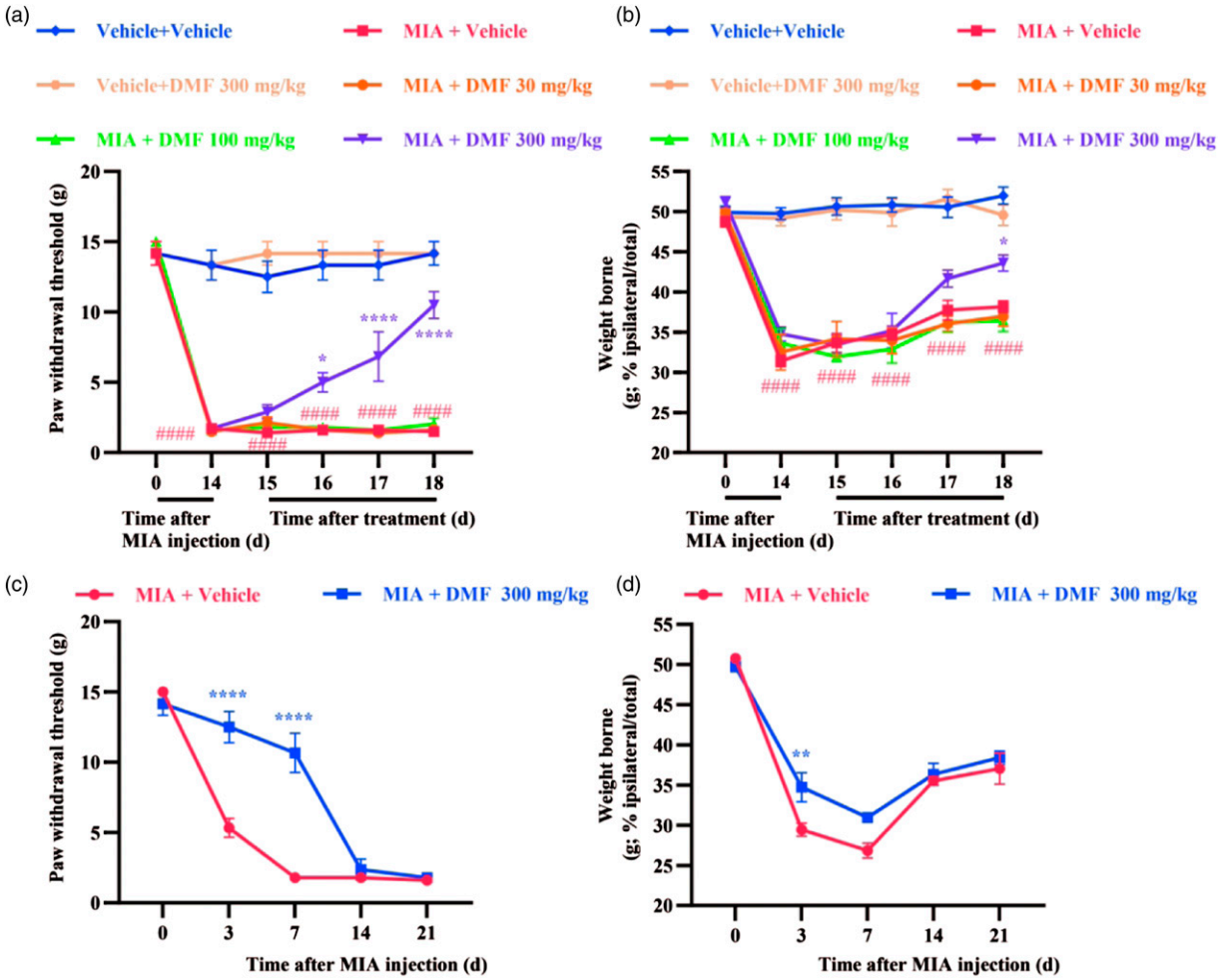

Figure 5. Analgesic effect of Nrf2 activator attenuated pain-related behaviors in a rat model OA. (A) Repetitive oral administration of dimethyl fumarate (300 mg/kg) remarkably reversed the mechanical paw withdrawal threshold in MIA + DMF 300 mg/kg group. However, compared with vehicle-treated rats, mechanical allodynia had no change in the 30 mg/kg and 100 mg/kg groups. (*$p < 0.05$, ****$p < 0.0001$ vs. MIA + Vehicle group, $^{\#\#\#\#}p < 0.0001$ vs. Vehicle + Vehicle group, $n = 6$ rats/group). (B) Weight-bearing asymmetry was markedly reversed at day 18 in MIA + DMF 300 mg/kg group. In the 30 mg/kg and 100 mg/kg groups, weight-bearing asymmetry had no change compared with vehicle-treated rats. (*$p < 0.05$ vs. MIA + Vehicle group, $^{\#\#\#\#}p < 0.0001$ vs. Vehicle + Vehicle group, $n = 6$ rats/group). (C) The MPWT was markedly upregulated from day 3 to day 7 in DMF-treated OA rats compared with vehicle-treated OA rats (****$p < 0.0001$ vs. MIA + Vehicle group, $n = 6$ rats/group). (D) Weighting-bearing asymmetry was also upregulated at day 3 (** $p < 0.01$ vs. MIA + Vehicle group, $n = 6$ rats/group).

group). Weighting-bearing asymmetry was also upregulated on day 3 (**$p < 0.01$ vs. MIA + Vehicle group, $n = 6$ rats/group). These data show that dimethyl fumarate delayed pain-related behaviors onset induced by MIA.

Effect of Nrf2 activator on the spinal level of Nrf2 and spinal mitochondrial biogenesis

To clarify whether the Nrf2 activator affected the protein expression of Nrf2 and impaired mitochondrial biogenesis in OA rats, 300 mg/kg dimethyl fumarate was orally administered once a day from day 14 for 5 days in a row. The protein expression of Nrf2 was remarkably upregulated after orally administrated dimethyl fumarate (Figure 6(a), ****$p < 0.0001$ vs. Vehicle + Vehicle group, $^{\#\#\#\#}p < 0.0001$ vs. MIA + Vehicle group, $n = 6$ rats/group). The mtDNA copy number and the protein expressions of mitochondrial biogenesis markers were reversed by dimethyl fumarate treatment (Figure 6(b) to (e), *$p < 0.05$ vs. Vehicle + Vehicle group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$ vs. MIA + Vehicle group, $n = 6$ rats/group). Based on these results, dimethyl fumarate restored mitochondrial biogenesis in a rat model of OA and upregulated the expression of Nrf2.

Reversal analgesic effect of Nrf2 inhibitor in OA rats

To clarify whether trigonelline, an Nrf2 inhibitor, could abolish dimethyl fumarate's analgesic effect, 20 mg/kg trigonelline was intraperitoneally administrated 30 min

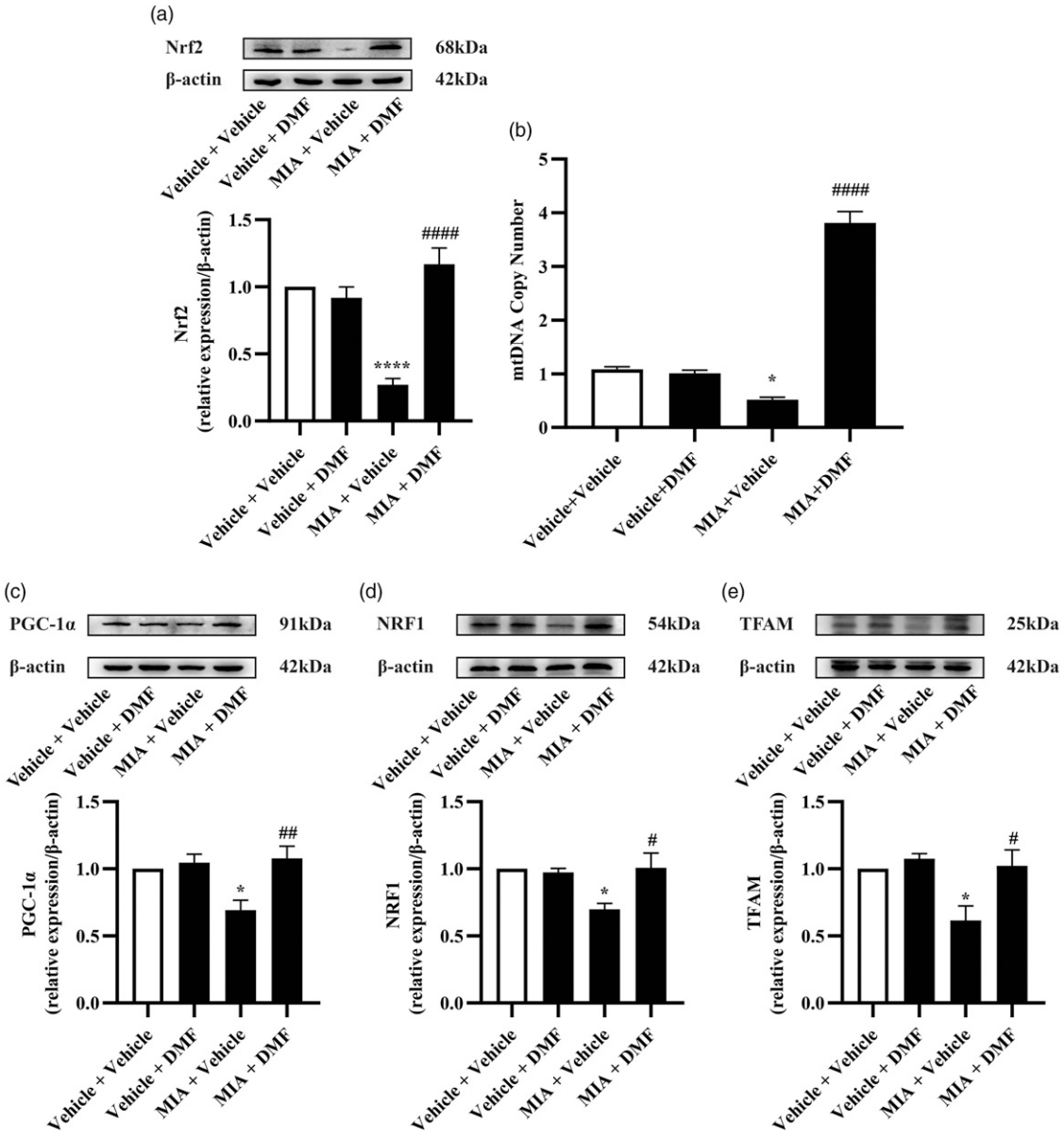

Figure 6. Effect of Nrf2 activator on the spinal level of Nrf2 and spinal mitochondrial biogenesis. (A) The protein level of Nrf2 was remarkably upregulated after orally administrated dimethyl fumarate (****$p < 0.0001$ vs. Vehicle + Vehicle group, #####$p < 0.0001$ vs. MIA + Vehicle group, $n = 6$ rats/group). (B–E) The mtDNA copy number and the expression of mitochondrial biogenesis markers were reversed by dimethyl fumarate treatment (*$p < 0.05$ vs. Vehicle + Vehicle group, #$p < 0.05$, ##$p < 0.01$, #####$p < 0.0001$ vs. MIA + Vehicle group, $n = 6$ rats/group).

before dimethyl fumarate. The behavioral tests were experienced before trigonelline administration and 2 h after dimethyl fumarate administration. As illustrated in Figure 7(a) and (b), the analgesic effect of dimethyl fumarate in OA rats was abolished by trigonelline (**$p < 0.01$, ****$p < 0.0001$ vs. MIA + Vehicle group, ##$p < 0.01$, ###$p < 0.001$, #####$p < 0.0001$ vs. MIA + DMF 300 mg/kg + Tri 20 mg/kg group, $n = 6$ rats/group). These data show that dimethyl fumarate alleviated pain-related behaviors by activating Nrf2.

Reversal effect of trigonelline on the spinal level of Nrf2 and spinal mitochondrial biogenesis

To clarify if in OA rats, the Nrf2 inhibitor could reverse the effect of dimethyl fumarate on the protein level of Nrf2 and mitochondrial biogenesis, the protein expressions were examined by western blot. Figure 8(a) to (d) illustrated that the western blot data suggested that trigonelline remarkedly reversed the effect that dimethyl fumarate affected mitochondrial biogenesis and the protein level of Nrf2 in a rat

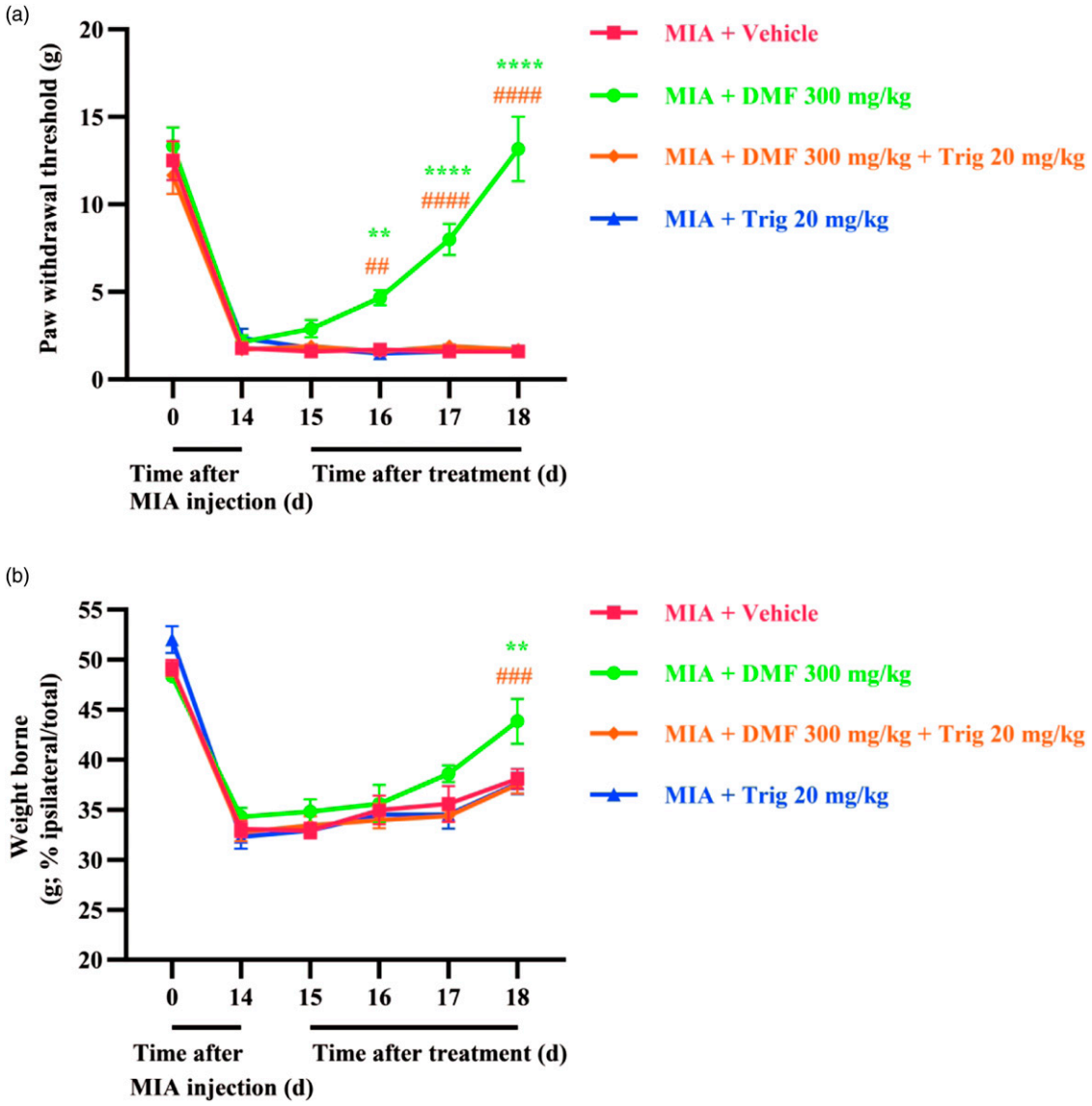

Figure 7. Reversal analgesic effect of Nrf2 inhibitor in OA rats. (A) The MPWT was remarkably reversed from day 16 to day 18 (** $p < 0.01$, **** $p < 0.0001$ vs. MIA + Vehicle group, $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$ vs. MIA + DMF 300 mg/kg + Tri 20 mg/kg group, $n = 6$ rats/group). (B) Weight-bearing asymmetry was reversed at day 18 (** $p < 0.01$ vs. MIA + Vehicle group, $^{\#\#\#}p < 0.001$ vs. MIA + DMF 300 mg/kg + Tri 20 mg/kg group, $n = 6$ rats/group).

model of OA (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ vs. MIA + Vehicle group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ vs. MIA + DMF 300 mg/kg + Tri 20 mg/kg group, $n = 6$ rats/group).

Discussion

Based on above results, we demonstrated that (1) mitochondrial dysfunction was involved in OA pain development; (2) the expression of Nrf2 was remarkably downregulated in OA rats; (3) dimethyl fumarate significantly alleviated pain behaviors and delayed the onset of pain behaviors in a rat model of OA; (4) dimethyl fumarate administration restored spinal mitochondrial biogenesis in OA rats; (5) the analgesic effect and mitochondrial biogenesis of dimethyl fumarate were reversed by trigonelline. Generally, these data show that dimethyl fumarate, an Nrf2 activator, alleviates pain behaviors in OA pain through activating Nrf2 and restoring mitochondrial biogenesis.

Consistent with our previous fundings, pain-related behaviors were rapidly onset and persisted until day 21 after injection of 1 mg of MIA.[14] Interestingly, the duration of the nadir is different between MPWT and weight-bearing asymmetry. Unlike MPWT, which remained at its lowest point from day 3 to day 21, weight-bearing asymmetry rapidly decreased, reaching its lowest point on day 3, and then

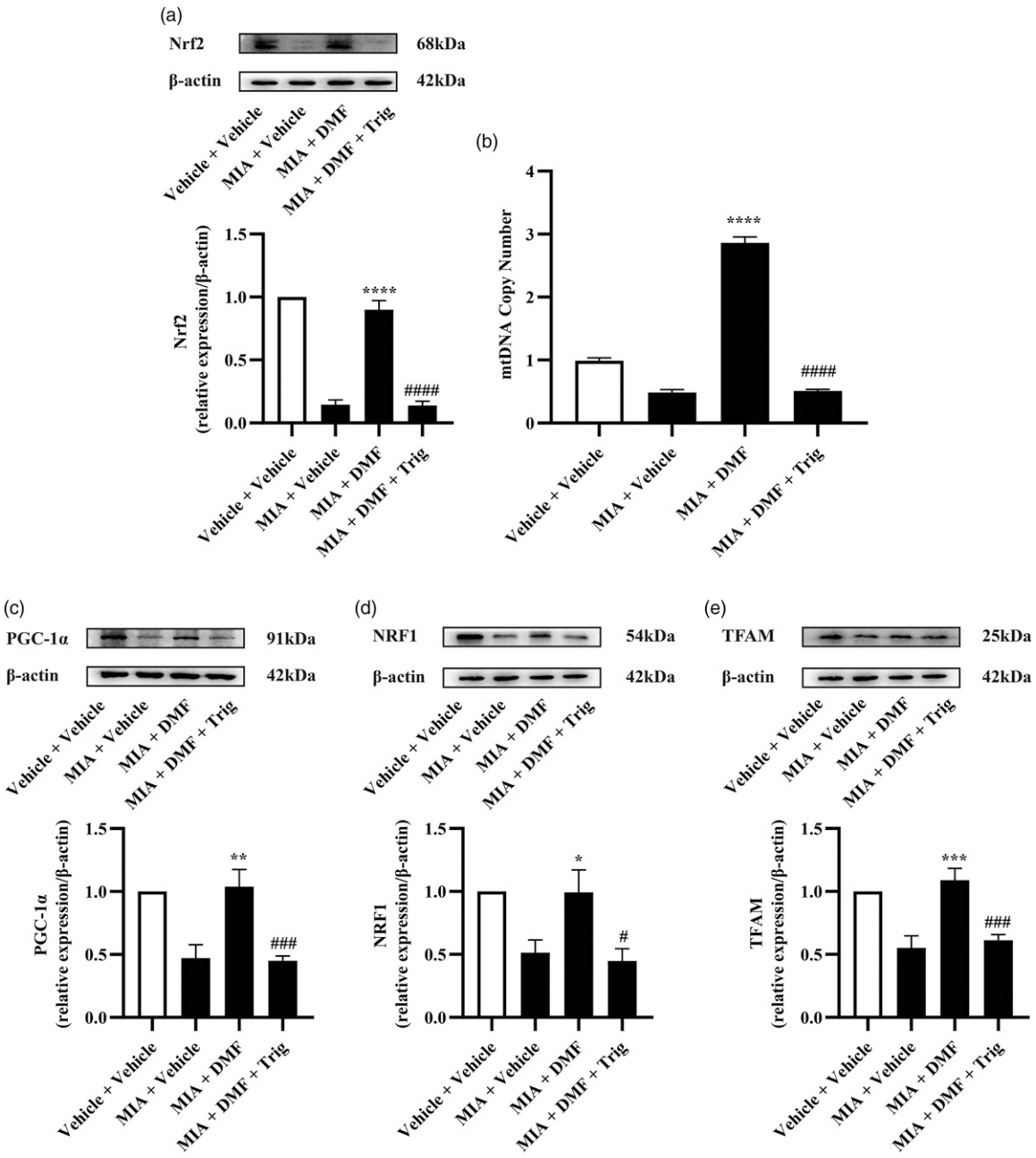

Figure 8. Reversal effect of trigonelline on the spinal level of Nrf2 and spinal mitochondrial biogenesis. (A) Trigonelline early treatment remarkedly reversed the effect of dimethyl fumarate on the protein level of Nrf2 (****$p < 0.0001$ vs. MIA + Vehicle group, $^{\#\#\#\#}p < 0.0001$ vs. MIA + DMF 300 mg/kg + Tri 20 mg/kg group, $n = 6$ rats/group). (B–D) Trigonelline early treatment remarkedly reversed the effect of dimethyl fumarate on the protein levels of PGC-1α, NRF1, and TFAM (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ vs. MIA + Vehicle group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$,$^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ vs. MIA + DMF 300 mg/kg + Tri 20 mg/kg group, $n = 6$ rats/group).

rose slightly. The underlying mechanisms may be that weight-bearing asymmetry is caused by central and peripheral sensitization whereas MPWT is caused by central sensitization.[31–33] A limitation of our study was that only male rats were used and no female rats were used. A recent study has shown that in spared nerve injury mice, dimethyl fumarate produced no analgesic effect in mice lacking Nrf2, regardless of sex.[27] Given the importance of sex differences in nociceptive hypersensitivity,[34,35] this question will be investigated in further studies.

Oxidative stress is caused by the excessive production of ROS.[36] Our previous study has shown that ROS scavengers could alleviate cancer-induced bone pain.[8] Oxidative stress leads to mitochondrial dysfunction, in turn, mitochondrial

dysfunction can produce more ROS.[37,38] Our past studies have suggested that spinal mitochondrial biogenesis impairment was present in neuropathic pain.[14–16] In this study, our results suggested that the mtDNA copy number was rapidly downregulated, as did the protein expressions of mitochondrial biogenesis markers. These data indicate that spinal mitochondrial biogenesis impairment was present in the rat model of OA.

Nrf2 plays a vital role in endogenous antioxidant defense. Our past studies have verified the importance of Nrf2 in chronic pain.[22,30] In this study, we used wetern blot to examine the protein expression of Nrf2 in the spinal cord. The spinal protein expression of Nrf2 was significantly downregulated which indicated that the antioxidant system was impaired in the rat model of OA. However, this result was different from our previous study. One possible explanation is that the experiments used different animal models. This shows that Nrf2 might have distinctive effects in special animal models, which needs further investigation. This data was similar to past studies which demonstrated that Nrf2 was significantly decreased in the rat model of paclitaxel-induced neuropathic pain.[39,40] Our behavioral tests indicated that repetitive administrations of dimethyl fumarate revered the established pain-related behaviors in rats with OA. This therapeutic effect of dimethyl fumarate was blocked by Nrf2 inhibitor trigonelline. Furthermore, early treatment with dimethyl fumarate from day 0-days 6 after MIA injection delayed the onset of pain-related behaviors in OA rats. Additionally, oral administration of dimethyl fumarate restored mitochondrial biogenesis and upregulated the expression of Nrf2. These results show that dimethyl fumarate, activating Nrf2, alleviated pain-related behaviors through restoring mitochondrial biogenesis. A limitation of this study was that dimethyl fumarate is not a special agonist of Nrf2, and the use of a special Nrf2 agonist and transgenic mouse models to investigate the development of OA pain and mitochondrial biogenesis might be more appropriate. Besides, dimethyl fumarate metabolizes and produces monomethyl fumarate which might affect the development of pain in the brain. Future studies will be addressed this question after oral administration of dimethyl fumarate. Furthermore, dimethyl fumarate has some side effects including flushing, nausea, and diarrhea in clinical trials.[23] Apart from flushing, gastrointestinal adverse effects are a frequent reason for patients to discontinue therapy with dimethyl fumarate.[41] However, in the present study, we didn't observe these adverse effects after drug administration in rats.

In summary, our study indicates that Nrf2 activation significantly alleviated mechanical allodynia through restoring mitochondrial biogenesis in a rat model of OA. These data indicate that dimethyl fumarate may provide an effective therapy for restoring mitochondrial biogenesis and attenuating pain behaviors induced by MIA.

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

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by grants from the National Natural Science Foundation of China (82071556, 81873793, 82001198, 82101310); and the National Key Research and Development Program of China (2020YFC2005303).

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