Heliyon 9 (2023) e17765

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Role of SIRT5 in the analgesic effectiveness of moxibustion at ST36 in mice with inflammatory pain

Chuan-yi Zuo [a], Chun-yan Gou [a,***], Cheng-shun Zhang [b], Xi Zhou [a], Peng Lv [b], Han-xiao Zhang [c], Zheng-peng Fan [a], Feng-wei Tian [a,**], Zhu-xing Wang [a,*]

[a] Department of Acupuncture, Chongqing Traditional Chinese Medicine Hospital, Chongqing 400021, Chongqing, China
[b] Acupuncture and Tuina School, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, Sichuan, China
[c] Faculty of Medicine, Université Paris-Saclay, Villejuif, 94800, France

ARTICLE INFO

Keywords:
Moxibustion
Analgesia
Inflammatory pain
SIRT5

ABSTRACT

Sirtuine5 (SIRT5) is an important molecule involved in the pathology of inflammatory diseases. To investigate the impact of SIRT5 on the analgesic effectiveness of moxibustion, we established a complete Freund's adjuvant- (CFA-) induced inflammatory pain in mice model. Moxibustion was applied at the Zusanli (ST36) acupoint in mice with inflammatory pain. The analgesic effectiveness was evaluated by thermal hyperalgesia and mechanical allodynia tests in the right paws after CFA injection. The expression of inflammatory cytokines, including the pro-inflammatory factors IL-1β and TNF-α, and the anti-inflammatory factors IL-4 and TGF-β expressions, was evaluated using by ELISA. Furthermore, SIRT5 was evaluated by immunofluorescence and western blotting. The results showed that, compared with the CFA group, both thermal and mechanical pain thresholds increased with moxibustion and the SIRT5 inhibitor MC3482 intervention at ST36. Additionally, compared to the CFA-induced group, the inflammatory mediators, including IL-1β and TNF-α, decreased, while the anti-inflammatory cytokines IL-4 and TGF-β increased with moxibustion and MC3482 ST36 acupoint injection. Western blot results showed a decreased expression of SIRT5 at the ST36 site with moxibustion and MC3482 injection, compared to the CFA-induced group. SIRT5 expression in the right paw of mice injected with moxibustion and MC3482 was higher than that in the CFA-induced group. This study revealed that SIRT5 expression is involved in moxibustion analgesia and may be a potential mediator in the regulation of analgesia.

1. Introduction

Pain is the most unbearable sensation that is driven by various stimuli such as irradiation, physical damage, microbial infection, and metabolic overload [1]. Currently, non-steroidal anti-inflammatory drugs (NSAIDs) are preferred for pain in the clinic [2]. The long-term use of NSAIDs cause serious health risks, including gastrointestinal and cardiovascular side reactions; new therapeutic targets are under exploration [3]. Recent evidence has revealed that both the immune and nervous systems are activated in response to

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: gcp666@126.com (F.-w. Tian), wzhuxing1963@163.com (Z.-x. Wang).

https://doi.org/10.1016/j.heliyon.2023.e17765
Received 18 June 2022; Received in revised form 17 June 2023; Accepted 27 June 2023
Available online 3 July 2023

pain by modulating calcium influx and the release of interleukins and neurotransmitters [4].

Sirtuins are a class of evolutionarily conserved nicotinamide adenine dinucleotide (NAD+)-dependent enzymes, which are composed of seven sirtuin paralogs (SIRT1-7) with divergent terminal primary structures responsible for different subcellular localizations [5]. NADH and its oxygenated NAD + are sensitive sensors of mitochondrial metabolism and calcium influx in cells, which plays a pivotal role in balancing the nervous and immune systems [6]. Calcium oscillations influence NF-κB interleukins expression [7]. As a member of the sirtuins family, Sirtuin 5 (SIRT5) is located in both the mitochondria and cytosol [8]. Large-scale proteomic analysis has proven that SIRT5 acts as a global regulator of lysine succinylation, malonylation, and glutarylation and plays a critical role in cellular homeostasis, including glycolysis, the tricarboxylic acid cycle, fatty acid oxidation, and nitrogen metabolism [9,10]. Evidence has now accumulated to document the roles of SIRT5 as a significant regulator in diseases, such as colitis, rheumatoid arthritis (RA), and sepsis, which has been suggested to be a potential target in regulating inflammatory diseases [11–13]. The deficiency of SIRT5 enhances the severity of inflammation by promoting TNF-α and IL-1β release in macrophages [11,12].

Moxibustion is a part of external treatment in Traditional Chinese Medicine and is considered as one of the possible alternative treatments with fewer adverse effects [14]. This strategy has been used for analgesia since ancient times and has been proven to be an effective and safer therapy than NSAIDs [15]. However, the mechanism by which moxibustion elevates pain is complicated. Recent studies have mainly focused on regulating the immune function, adjusting metabolism, and modulating neuro-related factors [16–21].

The immune system and metabolism play a major role in the pathogenesis of pain analgesic mechanism of moxibustion. SIRT5, an important mediator of immunity and metabolism, has attracted our attention. In this study, we established a complete Freund's adjuvant (CFA)-induced inflammatory pain model to investigate the role of SIRT5 in moxibustion analgesia.

2. Materials and methods

2.1. Animal

Adult male C57BL/6J mice, weighing 22 ± 2 g, were purchased from Shanghai Model Organisms Co., Ltd. All mice were kept on a 12-h light/dark cycle at 24 °C, and 40–50% humidity, with free access to food and water. After being adaptively fed for one week, mice were randomly divided into control, CFA, CFA + Moxi (moxibustion), CFA + MC3482 (MC3482 injected at ST36 acupoint), and CFA + MC3482+Moxi groups. After all the interventions, the mice were inhaled ethyl ether for narcosis and then were subjected to cervical dislocation. All animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the ethical guidelines of the International Association for the Study of Pain [22]. All experimental procedures were approved by the Animal Care and Use Committee of Chengdu University of Traditional Chinese Medicine (reference number: AECCDUTCM-2018-11).

2.2. CFA-induced inflammatory pain model

The inflammatory pain model was established by injecting 20 μL complete Freund's adjuvant (CFA) (Sigma-Aldrich, St. Louis, MO, USA) into the plantar surface of the right hind paw [23]. Local swelling and behavioral disability appeared within 24 h. Mice in the control group were injected with 20 μL normal saline.

2.3. Moxibustion intervention

Moxibustion was carried out on the fourth day of the CFA-induced pain model after CFA injection. Before moxibustion intervention, the fur was located at moxibustion site-ST36 (*Zusanli* acupoint, located 2 mm lateral to the anterior tubercle of the tibia in the anterior tibial muscle and 4 mm distal to the knee joint lower point) was shaved to expose. The CFA + Moxi, and CFA + MC3482+Moxi groups were operated with moxa sticks (length: 120 mm, diameter: 5 mm, Nanyang Hanyi Moxibustion Technology Development Co., Ltd., China) and the distance between the skin of ST36 and the lighted moxa sticks was controlled within 1.5 cm. The intervention was given for 5 days in total.

2.4. MC3482 intervention

MC3482 (MedChemExpress, Co. Ltd) is a specific sirtuin5 (SIRT5) inhibitor. MC3482 ST36 acupoint injection was performed on the fourth day of the CFA-induced pain model. The CFA + MC3482 and CFA + MC3482 + Moxi groups were injected with MC3482 (25 μM, 20 μl) at site-ST36 after the fur was shaved [24]. The control, CFA, and CFA + Moxi groups were injected with 20 μl saline. The

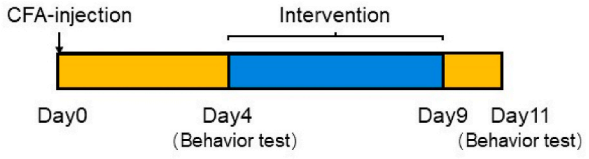

Fig. 1. Timeline for foot volumes and pain threshold measurements.

intervention was administered for five days (Fig. 1).

2.5. Behavior tests

The timeline for measuring pain thresholds is illustrated in Fig. 1. Before behavioral tests, mice acclimated to housing facilities for 5 days. Pain thresholds of the injured hind plantar were determined to reflect pain levels. Thermal hyperalgesia was assessed using radiant heat (PL-200, Techman, Chengdu, China), and thermal withdrawal latency (TWL) was defined as time mice removed hind paws from heat source. A 20s cut-off prevented potential tissue damage. Mechanical allodynia was evaluated by detecting paw mechanical withdrawal threshold with a dynamic plantar aesthesiometer (37450, UGO Basile, Germany) [25]. Mice were independently placed in the transparent acrylic box under the wire mesh bench, allowing access to the hind paw plantar surface. After 30 min acclimation, paw withdrawal threshold (PWT) was defined as the force to push mice to withdraw their hind paws. Each heat or force stimulus applied 3 times at 5 min intervals at each time point; the average threshold was taken.

2.6. ELISA assay

Mice were anesthetized with an overdose of chloral hydrate and euthanized by cervical dislocation. Tissue samples were carefully collected from the mice hind paws. In strict adherence to the manufacturer's guidelines, the concentrations of IL-1β, TNF-α, IL-4, and TGF-β in the paw tissues were precisely measured and quantified using ELISA kits. The ELISA procedures were followed meticulously to ensure accurate detection and reporting of the cytokine levels.

2.7. Immunofluorescence

Mice were anesthetized with an overdose of choral hydrate and with the cervical dislocation execution. The right ankle joint of mice was taken and samples collected were decalcified in 13% EDTA (pH 7.3), then placed in 30% sucrose overnight and embedded in OCT at −20 °C the next day. Frozen sections were cut (30 μm) and placed on glass micro slides coated with APS. Sections were fixed in 4% paraformaldehyde and incubated in a blocking solution containing 3% BSA, 0.02% Na N30.1%, and Triton X-100 in PBS for 2h at room temperature. After blocking, sections were incubated with the appropriate primary antibodies in a blocking solution at 4 °C overnight. The primary antibodies used were: anti-SIRT5 (1:400) from ProteinTech. The secondary antibody was a goat anti-rabbit (1:500) antibody (ProteinTech Group, Chicago, IL, USA). Slides were mounted with coverslips and visualized using a fluorescence microscope (CKX41 with an Olympus U-RFLT50 Power Supply Unit; Olympus, Tokyo, Japan). Image-pro plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) was applied to analyze the integrated optic density (IOD) and pixel area (AREA), and calculated the average optical (AO), AO = IOD/AREA.

2.8. Western blotting

The tissues were immediately excised to extract proteins. Sampled proteins were prepared by adding a lysis buffer containing 250 mM NaCl, 50 mM Tris–HCl pH 7.4, 50 mM sodium F, 1%NP-40, 0.02%Na N3, 5 mM EDTA, 1 mM Na3VO4, and 1 × protease inhibitor cocktail (AMRESCO, Solon, OH, USA). After homogenization using a Bullet Blender homogenizer (Next Advance, NY, USA), the extracted proteins (30 μg per sample assessed by BCA protein assay) were subjected to 8% SDS-Tris glycine gel electrophoresis and transferred to a PVDF membrane. The membrane was blocked with 5% non-fat milk in TBS-T buffer (10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20), incubated with the primary antibody in TBS-T with 1% bovine serum albumin for 1 h at room temperature. After peroxidase-conjugated secondary antibody (1:5000) was used, the image intensities of specific bands were quantified using ImageJ software (Bethesda, MD, USA). Antibodies used in the experiments were: anti-β-ACTIN (ProteinTech Group, Chicago, IL, USA), and anti-SIRT5 (ProteinTech Group, Chicago, IL, USA).

2.9. Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical evaluation was carried out by GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) to conduct analyses. Thermal threshold measurement and paw withdraw latency results were evaluated by repeated-measures two-way ANOVA followed by Bonferroni's post hoc test to make comparisons across groups over time. One-way ANOVA followed by the Fisher post hoc test for multiple comparisons or by unpaired *t*-test for comparisons for comparisons between two groups was employed for biochemical data to detect differences. In all cased $p < 0.05$ was considered to be significant.

3. Results

3.1. Moxibustion and MC3482 injection at ST36 relieved CFA-induced hypersensitivity in Hindpaws

The pain threshold, after three days of CFA stimuli, of paw withdrawal latency (PWL) and thermal withdrawal latency (TWL) in the CFA, CFA + Moxi, CFA + MC3482, and CFA + Moxi + MC3482 groups was significantly lower than that in control group ($p < 0.01$) (Fig. 2). After five days of intervention, with a day off, the PWL and TWL tests were given on day 11. Both PWL (Fig. 2A) and TWL

(Fig. 2B) scores increased (compared with the CFA group, $p < 0.05$) with moxibustion and MC3482 intervention at ST36.

Compared with the control group data (n = 12), PWL (A) and TWL (B) scores significantly decreased ($p < 0.01$) with CFA intervention in the CFA (n = 12), CFA + Moxi (n = 12), CFA + MC3482 (n = 12), and CFA + MC3482 + Moxi (n = 12) groups. The scores increased after the intervention in the CFA + Moxi, CFA + MC3482, and CFA + MC3482 + Moxi groups in comparison with those in model group ($p < 0.05$). *$p < 0.05$, in the CFA group; ▲$p < 0.01$, and in the control group.

3.2. Inflammatory cytokines changes within Moxi and MC3482 intervention

To identify the changes in inflammatory cytokines in the right paws induced by Moxi and MC3482, we measured the levels of inflammatory cytokines, including pro-inflammatory cytokines IL-1β and TNF-α, and anti-inflammatory cytokines IL-4 and TGF-β using ELISA (Fig. 3). Compared to the blank control, there was a significant increase in the levels of IL-1β (Fig. 3A) and TNF-α (Fig. 3B); meanwhile, the levels of TGF-β (Fig. 3D) decreased in the CFA group ($p < 0.05$). Compared to the CFA group, the levels of the inflammatory cytokine IL-1β decreased in the CFA + Moxi, CFA + MC3482, and CFA + MC3482 + Moxi groups ($p < 0.05$), and there were no statistical differences among the CFA + Moxi, CFA + MC3482, and CFA + MC3482 + Moxi groups in comparison with the control group ($p > 0.05$) (Fig. 3A). Additionally, compared to the CFA group, the level of TNF-α decreased in the CFA + Moxi, CFA + MC3482, and CFA + MC3482 + Moxi groups ($p < 0.05$); these expressions were still higher than those in the control group ($p < 0.05$) (Fig. 3B). Concerning anti-inflammatory cytokines, the level of IL-4 increased in the CFA + Moxi and CFA + MC3482 + Moxi groups compared to that in the CFA group ($p < 0.05$), and no statistical difference was observed among the control, CFA, and CFA + MC3482 groups ($p > 0.05$) (Fig. 3C). Compared to the CFA group, TGF-β expression was significantly higher in the control, CFA + Moxi, CFA + MC3482, and CFA + MC3482 + Moxi groups ($p < 0.05$) (Fig. 3D).

Pro-inflammatory cytokines IL-1β and TNF-α expression increased in the CFA group (n = 8) compared to the control group (n = 8) (*$p < 0.05$), and went down with Moxi (n = 8) and MC3482 (n = 8) intervention. Compared with CFA group, anti-inflammatory cytokines IL-4 and TGF-β increased in the CFA + Moxi (n = 8) and CFA + MC3482+Moxi (n = 8) groups. *$p < 0.05$, vs CFA group, #$p < 0.05$, vs control group.

3.3. SIRT5 expression at the stimulated sites of ST36 with moxibustion

Western blotting results showed that, compared with the control group, SIRT5 expression slightly increased in the CFA group, but there was no statistical difference between these two groups ($p > 0.05$). Compared to the blank control, SIRT5 expression decreased in CFA + MC3482, CFA + MC3482 + Moxi groups ($p < 0.05$). In comparison to the CFA group, SIRT5 expression was significantly reduced in the CFA + Moxi, CFA + MC3482, CFA + MC3482 + Moxi groups ($p < 0.05$) (Fig. 4). These results implied that both stimulating Moxi and MC3482 decreased SIRT5 expression at the ST36 stimulated site.

The control group (n = 5), CFA inflammatory pain model (n = 5), CFA + Moxi inflammatory pain with moxibustion group (n = 5), CFA + MC3482 inflammatory pain with MC3482 ST36 site injection group (n = 5), CFA + MC3482 + Moxi inflammatory pain with MC3482 ST36 site injection and moxibustion stimulation group (n = 5). *$p < 0.05$, compared with the CFA group. #$p < 0.05$, vs control group.

3.4. SIRT5 expression at the right hind paws of mice with moxibustion

The results from immunohistochemistry showed that SIRT5 immunoreactivity in the CFA + Moxi, CFA + MC3482, and CFA + MC3482 + Moxi groups was higher than that in the CFA group ($p < 0.05$) (Fig. 5A and B). Western blotting was performed to further quantify SIRT5 expression (Fig. 6A). The results showed that SIRT5 expression in the CFA group was dramatically lower than that in the control group ($p < 0.05$) (Fig. 6). After moxibustion and MC3482 intervention, compared to the CFA group, the expression of SIRT5 was enhanced in the CFA + Moxi, CFA + MC3482, CFA + MC3482 + Moxi groups ($p < 0.05$) (Fig. 6B).

The control group (n = 5), CFA inflammatory pain model (n = 5), CFA + Moxi inflammatory pain with moxibustion group (n = 5),

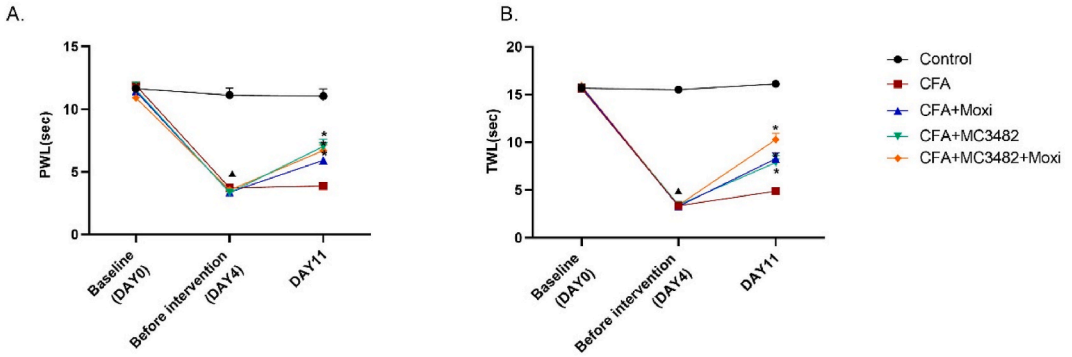

Fig. 2. Pain threshold changes of mice with moxibustion and MC3482 intervention at ST36 on inflammatory pain.

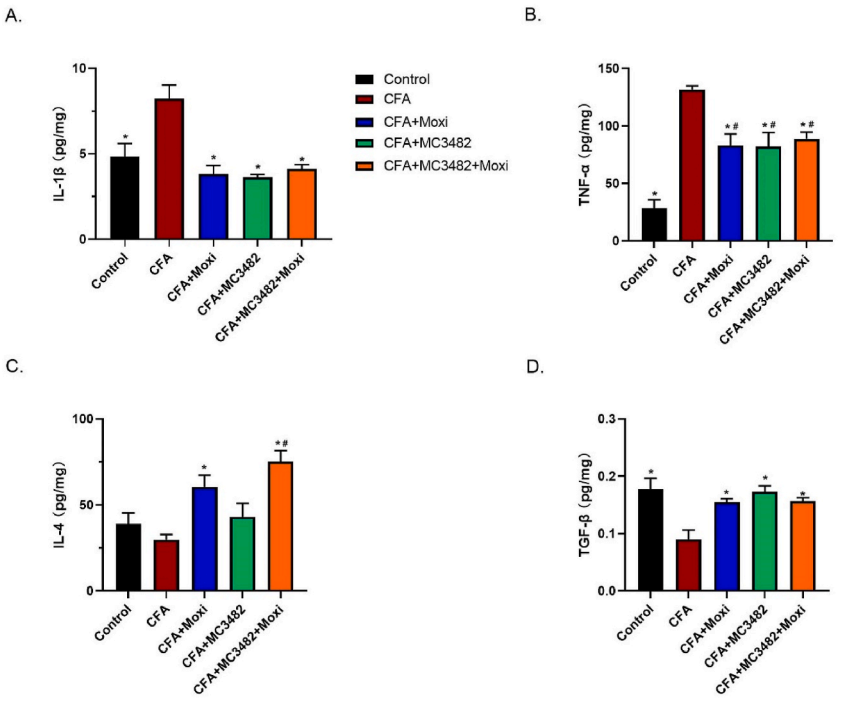

Fig. 3. Inflammatory cytokines expression intervened by moxibustion and MC3482.

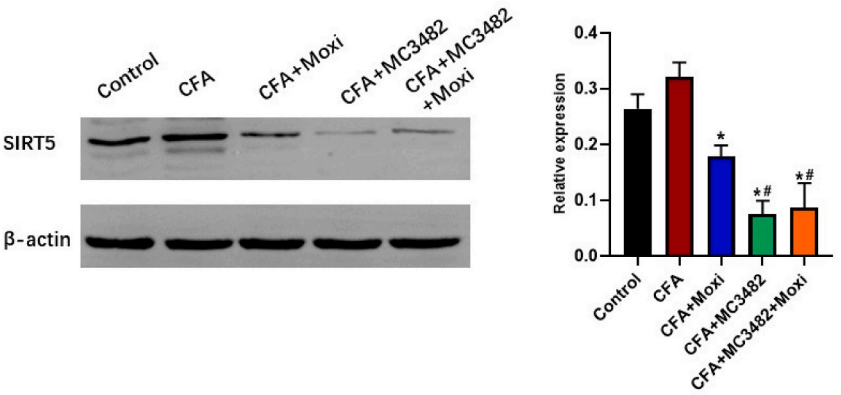

Fig. 4. Moxi and MC3482 decreased SIRT5 expression at ST36 skin of mice with CFA-induced pain tested by Western blotting.

CFA + MC3482 inflammatory pain with MC3482 ST36 site injection group (n = 5), CFA + MC3482 + Moxi inflammatory pain with MC3482 ST36 site injection and moxibustion stimulation group (n = 5). A. Representative immunohistochemistry image of SIRT5 expressions. B. Statistical analyze of SIRT5 immunoreactivity among these five groups. *$p < 0.05$, compared with the CFA group. Scale bars 20 μm.

The control group (n = 5), CFA inflammatory pain model (n = 5), CFA + Moxi inflammatory pain with moxibustion group (n = 5), CFA + MC3482 inflammatory pain with MC3482 ST36 site injection group (n = 5), CFA + MC3482 + Moxi inflammatory pain with MC3482 ST36 site injection and moxibustion stimulation group (n = 5). A. Representative Western blot image of SIRT5 expressions. B. Statistical analyze of SIRT5 expressions among these five groups. *$p < 0.05$, compared with the CFA group.

4. Discussion

Evidence demonstrated that moxibustion is an effective methods for treating inflammatory pain [26–28]. Therefore, in this study, a CFA-induced inflammatory pain model was established and the ST36 acupoint was selected as the site for moxibustion and injection of the SIRT5 inhibitor MC3482. Given the fact that ST36 is a significant acupoint in pain management, according to the traditional Chinese medicine theory, and recent modern research evidence [29–32]. Our results revealed that injecting MC3482 independently or

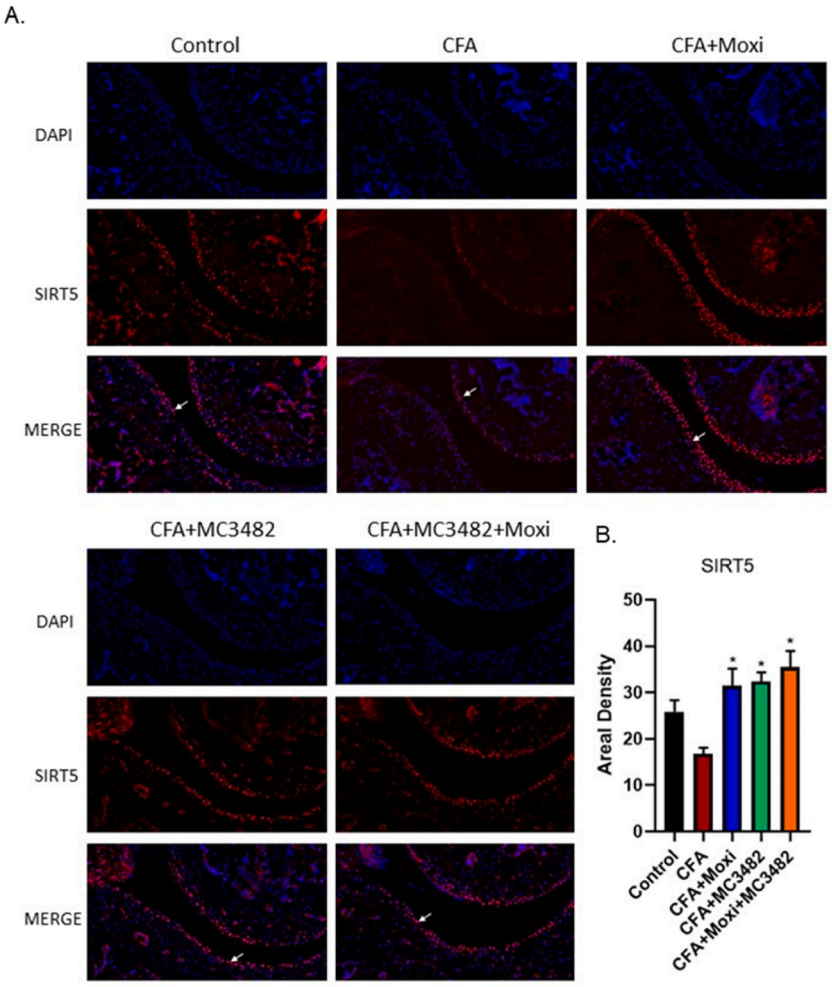

Fig. 5. Moxi and MC3482 enhanced SIRT5 expression at the foot of mice with CFA-induced pain tested by immunofluorescence.

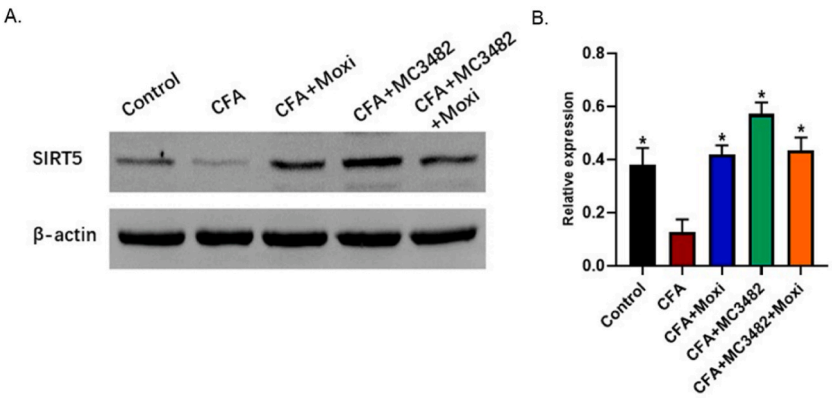

Fig. 6. Moxi and MC3482 enhanced SIRT5 expression at the foot of mice with CFA-induced pain tested by Western blotting.

in combination with moxibustion at ST36 improved the pain threshold in CFA-induced mice. There were no statistical differences among the three intervened groups, while there was a tendency that the moxibustion combined with MC3482 group had better thermal analgesia compared to groups using moxibustion or MC3482 independently. These results indicate that inhibition of SIRT5 expression at ST36 is an effective method of pain relief and suggest that SIRT5 may participate in the moxibustion analgesia mechanism.

Inflammatory mediators activated by immune cells are also thought to contribute to CFA-induced inflammatory pain [33,34]. Pro-inflammatory cytokines, such as IL-1β and TNF-α, are positively associated with the pathological process of pain, whereas anti-inflammatory cytokines, such as IL-4 and TGF-β, result in resolving inflammation and diminishing pain [35,36]. Pro-inflammatory cytokines, including IL-1β and TNF-α, are released when inflammation occurs and are mainly expressed in the peripheral blood and affected tissues [37]. Evidence suggests that utilizing intraplanar CFA induces mechanical and thermal hypersensitivity [50–53], and excites IL-1β and TNF-α release [38–40]. Our results demonstrates that IL-1β and TNF-α expression is accelerated in CFA-induced mice and restrained by moxibustion and MC3482 ST36 acupoint injections. IL-4 and TGF-β are important anti-inflammatory cytokines with diverse roles, such as wound repair, M2 macrophages activations, and T-cell proliferation [41,42]. After the initial inflammatory phase subsides, TGF-β has an important impact on tissue regeneration and inhibition of proinflammatory functions [43]. This suggests that reducing pro-inflammatory mediators and increasing anti-inflammatory cytokines may facilitate inflammatory pain recovery. Moreover, the expression level of TGF-β increased substantially with moxibustion and MC3482 ST36 acupoint injection, both independently and in combination, compared to that in CFA-induced mice. IL-4 signaling decreases the production and activity of several pain-related pro-inflammatory cytokines, such as IL-1β, COX-2, and NLRP3 [37]. Recent studies reported that IL-4 dampen the proinflammatory cytokine response by mediating macrophages to continuously produce opioid peptides and ameliorate pain [44]. These results showed that IL-4 expression was slightly increased when the SIRT5 inhibitor was applied to ST36 independently. While when combined with moxibustion, IL-4 expression increased significantly. Previous evidence revealed that IL-4 and SIRT5 are important regulators of pain relief, in the regulation of the macrophage immune response. Evidence demonstrated that these two regulators can act as initial mediators by transferring pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages to reduce pain [11,44,45]. Thus, this experiment suggests that the inflammatory response due to inflammatory pain could be relieved by moxibustion and SIRT5 inhibition at ST36. The results also implied the possibility that SIRT5 participated in pain relief via IL-4 in macrophages as an important mechanism of moxibustion, which needs further investigation.

MC3482 is a specific SIRT5 inhibitor that reduces SIRT5 expression at ST36. SIRT5 belong to the sirtuin family, and is involved in a complication, coordinated interplay between energy metabolism and genome stability and acts as a redox sensor to maintain cellular homeostasis [46]. SIRT5 has recently emerged as one of the most researched sirtuin family members. This is probably due to the absence of particular phenotypes or striking dysmetabolism in SIRT5-null mice under basal conditions [5]. SIRT5 primarily resides in the mitochondria and plays a distinct role in desuccinylase, deglutarylase, and demalonylase activities in the mitochondria [47]. Previous studies reported that tissues with high metabolic activity, such as the brain and muscle, exhibit high expression levels of SIRT5, which regulates protein substrates that are involved in glycolysis, electron transport chain, TCA cycle, ROS detoxification, ketone body formation, fatty acid β-oxidation, and nitrogenous waste management among other processes [48,49].

Additionally, we detected the expression of SIRT5 in the tissues at the ST36 acupoint and in the right paw of mice. These results showed that the expression of SIRT5 at the ST36 acupoint skin decreased after both moxibustion and MC3482 intervention. SIRT5 expression increased in the foot of mice with moxibustion and MC3482 intervention. MC3482 is a specific SIRT5 inhibitor with no effects on other sirtuin activities. Previous evidence suggested that SIRT5 participates in inflammatory diseases, including arthritis, lung fibrosis, aging, obesity, and colitis [50–52]. Our researches demonstrated that SIRT5 blocked IL-1β production in macrophages to prevent DSS-Induced Colitis in Mice [11,45]. SIRT5 overexpression plays as an anti-inflammatory role in TNF-α-induced inflammation [53]. The difference between our research and previous reports is that we found that inhibition of SIRT5 expression at ST36 could improve pain perception in CFA-induced mice by increasing SIRT5 expression in the paws. Notably, MC3482 is a specific SIRT5 inhibitor, this implies that the downregulation of SIRT5 expression at ST36 dampens the analgesic effect of moxibustion.

ST36 is an important acupoint in pain management according to the traditional Chinese medicine theory since ancient times [54]. Modern research has revealed that applying electroacupuncture at ST36 instead of at other acupoints drives the vagal–adrenal anti-inflammatory axis in mice [55]. These studies imply that the acupoint itself has specificity. Similarly, researchers found that applying downregulated anti-inflammatory mediators, such as caffeine or upregulated pro-inflammatory mediators, such as adenosine triphosphate at ST36 stimulated analgesic effectiveness [56,57]. Previous studies have showen that SIRT5 acts as an anti-inflammatory regulator. In this study, although SIRT5 expression was blocked at the ST36 acupoint, its expression in the right paw was enhanced, which is consistent with previous anti-inflammatory and pain relief roles of SIRT5; while further studies are needed to reveal the mechanism of the role of SIRT5 in moxibustion analgesia [12,58].

This study showed that SIRT5 expression is involved in moxibustion analgesia, but much research is still required to elucidate how the inhibition of SIRT5 at ST36 increases SIRT5 expression in the paw.

5. Conclusion

In conclusion, these findings show that moxibustion at ST36 could achieve analgesia and mediate pro- and anti-inflammatory mediators. SIRT5 participates in moxibustion analgesia.

Author contribution statement

Chuan-yi Zuo: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Feng-wei Tian: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Zhu-xing Wang: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Han-xiao Zhang, Zheng-peng Fan, Xi-Zhou, Peng Lv: Performed the experiments.
Cheng-shun Zhang, Chun-yan Gou: Performed the experiments; Analyzed and interpreted the data.

Funding statement

Chuanyi Zuo was supported by National Natural Science Foundation of China [82105010], Chongqing Postdoctoral Science Foundation [cstc2021jcyj-bshX0124].
Zhu-xing Wang was supported by Acupuncture and Chronobiology Key Laboratory of Sichuan Province Open Project [2021006].

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17765.

References

[1] P. Conaghan, A. Cook, J. Hamilton, P. Tak, Therapeutic options for targeting inflammatory osteoarthritis pain, Nat. Rev. Rheumatol. 15 (6) (2019) 355–363, https://doi.org/10.1038/s41584-019-0221-y.
[2] M. Tomić, U. Pecikoza, A. Micov, S. Vučković, R. Stepanović-Petrović, Antiepileptic drugs as analgesics/adjuvants in inflammatory pain: current preclinical evidence, Pharm. Therap. 192 (2018) 42–64, https://doi.org/10.1016/j.pharmthera.2018.06.002.
[3] B. Botz, K. Bölcskei, Z. Helyes, Challenges to develop novel anti-inflammatory and analgesic drugs, Wiley Interdisc. Rev. Nanomed. Nanobiotech. 9 (3) (2016).
[4] J. Liu, J. Yu, C. Cheung, Immune actions on the peripheral nervous system in pain, Int. J. Mol. Sci. 22 (3) (2021), https://doi.org/10.3390/ijms22031448.
[5] G. Di Emidio, et al., Mitochondrial sirtuins in reproduction, Antioxidants 10 (7) (2021), https://doi.org/10.3390/antiox10071047.
[6] R. Marcu, B. Wiczer, C. Neeley, B. Hawkins, Mitochondrial matrix Ca^{2+} accumulation regulates cytosolic NAD^{+}/NADH metabolism, protein acetylation, and sirtuin expression, Mol. Cell Biol. 34 (15) (2014) 2890–2902, https://doi.org/10.1128/mcb.00068-14.
[7] L. Zhu, et al., Cumulated Ca2⁺ spike duration underlies Ca2⁺ oscillation frequency-regulated NFκB transcriptional activity, J. Cell Sci. 124 (2011) 2591–2601, https://doi.org/10.1242/jcs.082727.
[8] M. Rardin, et al., SIRT5 regulates the mitochondrial lysine succinylome and metabolic networks, Cell Metabol. 18 (6) (2013) 920–933, https://doi.org/10.1016/j.cmet.2013.11.013.
[9] J. Guan, et al., Sirtuin 5 regulates the proliferation, invasion and migration of prostate cancer cells through acetyl-CoA acetyltransferase 1, J. Cell Mol. Med. 24 (23) (2020) 14039–14049, https://doi.org/10.1111/jcmm.16016.
[10] Y.Q. Wang, et al., Sirtuin5 contributes to colorectal carcinogenesis by enhancing glutaminolysis in a deglutarylation-dependent manner, Nat. Commun. 9 (1) (2018) 545.
[11] F. Wang, et al., SIRT5 desuccinylates and activates pyruvate kinase M2 to block macrophage IL-1β production and to prevent DSS-induced colitis in mice, Cell Rep. 19 (11) (2017) 2331–2344, https://doi.org/10.1016/j.celrep.2017.05.065.
[12] N. Zhang, et al., Sirtuin 5 deficiency increases disease severity in rats with adjuvant-induced arthritis, Cell. Mol. Immunol. 17 (11) (2020) 1190–1192, https://doi.org/10.1038/s41423-020-0380-4.
[13] K. Qin, et al., NAD dependent deacetylase Sirtuin 5 rescues the innate inflammatory response of endotoxin tolerant macrophages by promoting acetylation of p65, J. Autoimmun. 81 (2017) 120–129, https://doi.org/10.1016/j.jaut.2017.04.006.
[14] C. Zhang, et al., The role of STIM1/ORAI1 channel in the analgesic effect of grain-sized moxibustion on inflammatory pain mice model, Life Sci. 280 (2021), 119699, https://doi.org/10.1016/j.lfs.2021.119699.
[15] Q. Huang, H. Wu, J. Liu, Y. Hong, S. Wang, Bibliometric analysis of diseases spectrum of moxibustion therapy, J. Acupunc. Tuina Sci. 10 (6) (2012) 7.
[16] S. Pan, et al., Moxibustion for primary dysmenorrhea: an adjuvant therapy for pain relief. Evidence-Based complementary and alternative medicine, eCAM 2022 (2022), 6864195, https://doi.org/10.1155/2022/6864195.
[17] T. Lu, J. Zhang, Y. Lv, Y. Wu, The effect of warm needle moxibustion on lumbar disc herniation, Am. J. Tourism Res. 13 (5) (2021) 5059–5065.
[18] Q. Qi, et al., Moxibustion treatment modulates the gut microbiota and immune function in a dextran sulphate sodium-induced colitis rat model, World J. Gastroenterol. 24 (28) (2018) 3130–3144, https://doi.org/10.3748/wjg.v24.i28.3130.
[19] X. Yang, W. Wang, Y. Zhang, J. Wang, F. Huang, Moxibustion improves ovary function by suppressing apoptosis events and upregulating antioxidant defenses in natural aging ovary, Life Sci. 229 (2019) 166–172, https://doi.org/10.1016/j.lfs.2019.05.040.
[20] H. Yin, et al., Purinergic ATP triggers moxibustion-induced local anti-nociceptive effect on inflammatory pain model, Purinergic Signal. (2021), https://doi.org/10.1007/s11302-021-09815-5.
[21] M. Yuan, et al., Effects of heat-sensitive moxibustion combined with naprapathy and warming needle moxibustion combined with naprapathy in patients with periarthritis of shoulder, Am. J. Tourism Res. 13 (7) (2021) 7804–7811.
[22] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (2) (1983) 109–110.
[23] K. Nakamoto, et al., Hypothalamic GPR40 signaling activated by free long chain fatty acids suppresses CFA-induced inflammatory chronic pain, PLoS One 8 (12) (2013), e81563.
[24] L. Polletta, et al., SIRT5 regulation of ammonia-induced autophagy and mitophagy, Autophagy 11 (2) (2015) 253–270, https://doi.org/10.1080/15548627.2015.1009778.
[25] W. Zhou, et al., Analgesic Effect of Moxibustion with Different Temperature on Inflammatory and Neuropathic Pain Mice: A Comparative Study. Evidence-Based Complementary and Alternative Medicine : eCAM. 2017, 2017, 4373182, https://doi.org/10.1155/2017/4373182.

[26] J.H. Kim, H.K. Kim, Y.I. Park, I.C. Sohn, B.R. Park, Moxibustion at ST36 alleviates pain in complete Freund's adjuvant-induced arthritic rats, Am. J. Chin. Med. 34 (1) (2006) 57–67.
[27] Myeong Soo Lee, Tae-Young Choi, Kang *Jung Won, Leey *Beom-Joon, E. Ernstz, Moxibustion for treating pain: a systematic review, Am. J. Chin. Med. 38 (5) (2010) 829–838.
[28] C.S. Zhang, C.Y. Zuo, P. Lv, H.X. Zhang, X.Q. Dai, The role of STIM1/ORAI1 channel in the analgesic effect of grain-sized moxibustion on inflammatory pain mice model, Life Sci. 280 (379291) (2021), 119699.
[29] H. Luo, et al., Glucocorticoid receptor contributes to electroacupuncture-induced analgesia by inhibiting Nav1.7 expression in rats with inflammatory pain induced by complete freund's adjuvant, Neuromodulation : J. Int. Neuromodul. Soci. (2021), https://doi.org/10.1111/ner.13499.
[30] Y. Wang, et al., βEffect of moxibustion on -EP and dyn levels of pain-related indicators in patients with rheumatoid arthritis. Evidence-Based complementary and alternative medicine, eCAM 2021 (2021), 6637554, https://doi.org/10.1155/2021/6637554.
[31] R. Torres-Rosas, et al., Dopamine mediates the vagal modulation of the immune system by electroacupuncture, Nat. Med. 20 (3) (2014) 291–295.
[32] S. Liu, et al., A neuroanatomical basis for electroacupuncture to drive the vagal-adrenal axis, Nature (2021), https://doi.org/10.1038/s41586-021-04001-4.
[33] P. Baral, S. Udit, I. Chiu, Pain and immunity: implications for host defence, Nat. Rev. Immunol. 19 (7) (2019) 433–447, https://doi.org/10.1038/s41577-019-0147-2.
[34] B. Jiang, T. Liu, Y. Gao, Chemokines in chronic pain: cellular and molecular mechanisms and therapeutic potential, Pharm. Therap. 212 (2020), 107581, https://doi.org/10.1016/j.pharmthera.2020.107581.
[35] R. Domoto, F. Sekiguchi, M. Tsubota, A. Kawabata, Macrophage as a peripheral pain regulator, Cells 10 (8) (2021), https://doi.org/10.3390/cells10081881.
[36] M. Noack, P. Miossec, Importance of lymphocyte-stromal cell interactions in autoimmune and inflammatory rheumatic diseases, Nat. Rev. Rheumatol. (2021), https://doi.org/10.1038/s41584-021-00665-4.
[37] A. Vanderwall, E. Milligan, Cytokines in pain: harnessing endogenous anti-inflammatory signaling for improved pain management, Front. Immunol. 10 (2019) 3009, https://doi.org/10.3389/fimmu.2019.03009.
[38] M.K. Boettger, S. Hensellek, F. Richter, M. Gajda, H.G. Schaible, Antinociceptive effects of tumor necrosis factor α neutralization in a rat model of antigen-induced arthritis: evidence of a neuronal target, Arthritis Rheumatol. 58 (8) (2014) 2368–2378.
[39] T. Cunha, et al., A cascade of cytokines mediates mechanical inflammatory hypernociception in mice, Proc. Natl. Acad. Sci. U. S. A. 102 (5) (2005) 1755–1760, https://doi.org/10.1073/pnas.0409225102.
[40] A.D. Cook, A.D. Christensen, D. Tewari, S.B. Mcmahon, J.A. Hamilton, Immune cytokines and their receptors in inflammatory pain, Trends Immunol. 39 (3) (2018) 240–255.
[41] J. Prado, et al., Cytokine receptor clustering in sensory neurons with an engineered cytokine fusion protein triggers unique pain resolution pathways, Proc. Natl. Acad. Sci. U. S. A. 118 (11) (2021), https://doi.org/10.1073/pnas.2009647118.
[42] S. Hwang, G. Chung, Y. Kim, C. Park, The role of maresins in inflammatory pain: function of macrophages in wound regeneration, Int. J. Mol. Sci. 20 (23) (2019), https://doi.org/10.3390/ijms20235849.
[43] M. Frinchi, et al., Anti-inflammatory and antioxidant effects of muscarinic acetylcholine receptor (mAChR) activation in the rat hippocampus, Sci. Rep. 9 (1) (2019), 14233, https://doi.org/10.1038/s41598-019-50708-w.
[44] M. Celik, D. Labuz, J. Keye, R. Glauben, H. Machelska, IL-4 induces M2 macrophages to produce sustained analgesia via opioids, JCI Insight 5 (4) (2020), https://doi.org/10.1172/jci.insight.133093.
[45] P. Yao, T. Chen, P. Jiang, L. Li, W. Du, Functional skewing of TRIM21-SIRT5 interplay dictates IL-1β production in DSS-induced colitis, EMBO Rep. 23 (9) (2022), e54391, https://doi.org/10.15252/embr.202154391.
[46] P. Parihar, I. Solanki, M. Mansuri, M. Parihar, Mitochondrial sirtuins: emerging roles in metabolic regulations, energy homeostasis and diseases, Exp. Gerontol. 61 (2015) 130–141, https://doi.org/10.1016/j.exger.2014.12.004.
[47] M. Hirschey, Y. Zhao, Metabolic regulation by lysine malonylation, succinylation, and glutarylation, Mol. Cell. Proteomics : MCP. 14 (9) (2015) 2308–2315, https://doi.org/10.1074/mcp.R114.046664.
[48] S. Kumar, D. Lombard, Functions of the sirtuin deacylase SIRT5 in normal physiology and pathobiology, Crit. Rev. Biochem. Mol. Biol. 53 (3) (2018) 311–334, https://doi.org/10.1080/10409238.2018.1458071.
[49] M. Tan, et al., Lysine glutarylation is a protein posttranslational modification regulated by SIRT5, Cell Metabol. 19 (4) (2014) 605–617, https://doi.org/10.1016/j.cmet.2014.03.014.
[50] U. Das, "Cell membrane theory of senescence" and the role of bioactive lipids in aging, and aging associated diseases and their therapeutic implications, Biomolecules 11 (2) (2021), https://doi.org/10.3390/biom11020241.
[51] S. Mazumder, M. Barman, U. Bandyopadhyay, S. Bindu, Sirtuins as endogenous regulators of lung fibrosis: a current perspective, Life Sci. 258 (2020), 118201, https://doi.org/10.1016/j.lfs.2020.118201.
[52] K. Kalous, S. Wynia-Smith, S. Summers, B. Smith, Human sirtuins are differentially sensitive to inhibition by nitrosating agents and other cysteine oxidants, J. Biol. Chem. 295 (25) (2020) 8524–8536, https://doi.org/10.1074/jbc.RA119.011988.
[53] H. Yu, et al., Screening analysis of sirtuins family expression on anti-inflammation of resveratrol in endothelial cells, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. : Int. Med. J. Exper. Clin. Res. 25 (2019) 4137–4148, https://doi.org/10.12659/msm.913240.
[54] H. Huang, et al., zusanliBrain activities responding to acupuncture at ST36 in healthy subjects: a systematic review and meta-analysis of task-based fMRI studies, Front. Neurol. 13 (2022), 930753, https://doi.org/10.3389/fneur.2022.930753.
[55] S. Liu, et al., A neuroanatomical basis for electroacupuncture to drive the vagal-adrenal axis, Nature 598 (7882) (2021) 641–645, https://doi.org/10.1038/s41586-021-04001-4.
[56] K. Liu, et al., Caffeine attenuates electroacupuncture effect on pressure pain threshold and tolerance in healthy individuals: a randomized controlled trial, Front. Neurol. 13 (2022), 859624, https://doi.org/10.3389/fneur.2022.859624.
[57] L. Wang, et al., Activation of subcutaneous mast cells in acupuncture points triggers analgesia, Cells 11 (5) (2022), https://doi.org/10.3390/cells11050809.
[58] F. Molinari, et al., SIRT5 inhibition induces Brown fat-like phenotype in 3T3-L1 preadipocytes, Cells 10 (5) (2021), https://doi.org/10.3390/cells10051126.