Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Tetrandrine alleviates inflammation and promotes macrophage M2 polarization in gouty arthritis by NF-κB-mediated Lcp1

Li Fang[1#], Rong Shen[2#], Yao Lu[1], Xiangfeng Xu[1*], Fang Huang[3*]

[1] *Department of Rheumatology and Immunology, Zhoushan Hospital of Zhejiang Province, Zhoushan, Zhejiang 316021, China*
[2] *Department of Geriatrics, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China*
[3] *Department of Acupuncture, Zhoushan Hospital of Traditional Chinese Medicine, Zhoushan, Zhejiang 316000, China*

Article Info

Article history:

Received: September 24, 2023
Accepted: December 14, 2023
Published: February 29, 2024

Use your device to scan and read the article online

Abstract

Gouty arthritis (GA) is an inflammatory disease caused by the deposition of monosodium urate (MSU) crystals into joints. Tetrandrine (TET) is a bisbenzylisoquinoline alkaloid extracted from the root of *Stephania tetrandra* and can exert an anti-inflammatory function in different diseases. Nevertheless, the specific function of TET in GA remains unclear. We established the GA mouse model by MSU injection into joints of mice. Paw volume and gait score were detected for measuring the degree of joint swelling and the situation of joint dysfunction. Western blot were utilized to test the alterations of M1-related factors (IL-6, IL-1β, TNF-α, IL-12, and iNOS) and M2-related factors (Mgl1, Mgl2, Pgc1-β, Arg-1, and IL-10). The activity of NF-κB p65 in tissues was determined. The interaction of NF-κB p65 and Lcp1 was measured by ChIP and luciferase reporter assay. Lcp1 KO mice were utilized to detect the effect of Lcp1 depletion on GA process. TET treatment markedly suppressed MSU-induced joint swelling, joint dysfunction, and joint injury in GA mice. TET can also reduce inflammatory reactions in MUS-induced mice. Furthermore, we proved that TET facilitated M2 macrophage polarization and inhibited M1 macrophage polarization in GA mice. In addition, TET was found to inhibit NF-κB activity and NF-κB-mediated Lcp1 expression. Lcp1 knockdown can improve joint injury and promote M2 macrophage polarization in GA mice, while this effect was further enhanced by TET. TET alleviates inflammation and facilitates macrophage M2 polarization in GA by NF-κB-mediated Lcp1.

Keywords: Gouty arthritis, Lcp1, Macrophage polarization, NF-κB, Tetrandrine

1. Introduction

Gouty arthritis (GA) is an inflammatory disease caused by disorders of purine metabolism and decreased uric acid excretion [1]. GA is characterized by an innate immune disorder and causes a systemic inflammatory reaction which could result in the deposition of monosodium urate (MSU) crystals in the joints and surrounding tissues [2]. The incidence rate of GA is about 1-2% and is increasing every year, especially in developing countries [3]. It can cause assorted complications, such as hypertension, type 2 diabetes, coronary artery disease, and kidney disease [3]. GA is paroxysmal and commonly occurs in the human extremities, usually presenting as redness, swelling, warmth and pain in individual joints and limiting movement [4]. Current research on the subject suggests that episodes of GA are associated with people's diets and lifestyles, such as high protein, high purine compounds and high-stress lifestyles [4]. First-line medications commonly used in GA include NSAIDs, colchicine and glucocorticoids, which can quickly suppress the inflammatory response and relieve pain [5]. These western drugs have shown good efficacy in treating single episodes of GA, but repeated use tends to develop resistance and cause adverse effects such as gastrointestinal, liver and kidney damage, which leads to diminished utility and effectiveness of the drugs. Active extracts of natural herbs are increasingly receiving more and more attention because of their promising efficacy, less adverse effects and wide availability [6]. Therefore, the study of traditional herbal medicines is necessary to develop new GA treatments.

Macrophages are the crucial components of the immune system. Macrophage polarization has been confirmed by many studies to exert a crucial function in assorted pathophysiological processes, including inflammatory reaction, tissue repair and metabolism [7, 8]. Macrophages have two polarized phenotypes, the classically activated M1 and the alternatively activated M2. M1 macrophages play mainly pro-inflammatory, pathogenic microbial clearance and anti-tumor roles, whereas M2 macrophages play the opposite role, suppressing inflammatory responses, promoting tissue remodeling, and participating in immune regulation [9]. Studies have shown that macrophages acquire a pro-

* Corresponding author.
E-mail address: xuxf76@hotmail.com (X. Xu); hf229486859@163.com (F. Huang).
# These authors contributed equally
Doi: http://dx.doi.org/10.14715/cmb/2024.70.2.29

inflammatory M1 phenotype after MSU stimulation [10]. Then, endothelial cells are damaged by the activation of inflammatory mediators [11]. Studies have confirmed that Kinsenoside alleviates osteoarthritis via macrophage polarization through the inactivation of NF-κB/MAPK pathway [12]. Simiao Wan attenuates MSU-stimulated arthritis by regulating macrophage M2 polarization [13].

Tetrandrine (TET) is a bisbenzylisoquinoline alkaloid extracted from the root of *Stephania tetrandra* and possesses pharmacological actions such as anti-inflammation, analgesia, anti-fibrosis, and anti-tumor [14], which is widely utilized in human diseases such as tuberculosis, hyperglycemia, malaria, and cardiovascular diseases [14, 15]. Studies have demonstrated that TET can suppress the NF-κB pathway via the inhibition of IκBα and NF-κB p65 phosphorylation, thereby decreasing the release of proinflammatory factors [16]. TET suppresses rheumatoid arthritis via inhibiting neutrophil activity [17]. TET inhibits migration and invasion of rheumatoid arthritis fibroblast-like synoviocytes via activating PI3K signaling [18]. However, TET function in GA process remains unclear.

In this study, the MSU-induced GA mouse model was established, and we explored whether TET can regulate macrophage M2 polarization to alleviate GA process.

2. Materials and methods

2.1. Animals

All animal studies were approved by the Ethics Committee of YHANGZHOU HIBIO TECHNOLOGY CO. LTD (HB2208016). Lcp1 knock-out (KO) mice were purchased from Cyagen Biosciences Inc. (Guangzhou, China) and C57BL/6J mice were purchased from HANGZHOU HIBIO TECHNOLOGY CO. LTD (HB2208016). Mice were housed in the standard environment (23–25°C, 40–60% humidity, and a 12 h light/dark cycle) and adaptively raised for a week.

2.2. The establishment of MIA mice

Sixty C57BL/6J mice were randomly divided into 6 groups (n=10 per group): the control group, the MIA group, the colchicine (COL; 0.3 mg/kg) group, the 2 mg/kg TET group, the 4 mg/kg TET group, and the 8 mg/kg TET group. Mice in the control group and MIA group were intragastrically administered an equal volume of normal saline for 7 days. Mice in the COL group and TET groups received COL or TET (Sigma Aldrich, St. Louis, MO, USA) intragastrically once a day for 7 days. On day 6, mice of the MIA group, the COL group, and the TET group were subjected to intra-articular injection of MSU crystals (0.5 mg in 20 µl of sterile PBS; Sigma–Aldrich) into the left paw under 1% isoflurane anaesthesia. Mice in the control group mice were injected with an equal volume of PBS. After 24 h MSU, the paw volume of the left hind limb was assessed through an electronic caliper and the gait score was measured. Next, the mice were anesthetized with isoflurane and the blood was gathered. Then, mice were euthanized and the ankle joint tissues of mice were collected for further assays.

2.3. Measurement of gait score

Gait score was utilized for measuring the behavior disorder. It is divided into four grades from 0 to 3: 0 represents normal gait; 1 represents slight limp; 2 represents moderate limp; and 3 represents severe limp. The gait score was used by two observers who did not know the experimental protocol.

2.4. Enzyme-Linked Immunosorbent Assay (ELISA)

Serum was gathered by centrifugation at 6000 × g for 10 min. The contents of IL-1β and IL-10 in serum were determined by their corresponding ELISA kit (Solarbio, Inc., China) in line with user guides.

2.5. H&E staining assay

The ankle joint tissues were fixed with 4% paraformaldehyde, decalcified in 10% EDTA, and then embedded in paraffin. Next, they were sliced at 4 µm intervals. Sections were stained with Hematoxylin for 15 min and re-stained with Eosin for 5 min. Later, they were dehydrated with alcohol, hyalinized, and sealed with neutral resins. The microscope (Olympus) was applied for observation.

2.6. Detection of NF-κB activity

Based on the user guides, NE-PER nuclear extraction kit (Thermo Fisher, USA) was applied to obtain the nuclear extracts. Tissues were homogenized in the CER I buffer and cultured on ice. After 10 min, CER II buffer was supplemented into the tubes and subjected to centrifugation. The supernatants were removed and the pellets were resuspended in ice-cold nuclear extraction reagent. After centrifugation, the supernatant containing the nuclear extract was performed with NF-kB p65 Transcription Factor Assay Kit (Abcam) in accordance with user guides. The activity of NF-kB p65 in the sample was quantified by reading the absorbance value at 450 nm.

2.7. Cell culture and transfection

RAW264.7 macrophage cells and HEK-293T cells were obtained from Procell (Wuhan, China) and incubated in DMEM (Gibco, USA) added with 10% FBS (Hycolon, USA) at 37°C with 5% CO_2. To silence p65 expression, cells were transfected with 25 nM of p65 siRNA (si-p65; Genechem, Shanghai, China) and the negative control siRNA (si-NC; Genechem) by Lipofectamine 3000 (Invitrogen, USA) for 48 h.

2.8. RT-qPCR

TRIzol (Invitrogen) was used to extract RNA from cells or tissues. Reverse transcription was performed with ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan) in line with user guides. Next, qPCR was performed utilizing SYBR Green Real-time PCR Master Mix (Toyobo) on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Gene expression was calculated with the $2^{-\Delta\Delta}$Ct methods normalized to U6 or GAPDH.

2.9. Western blot

The total proteins from collected tissues were extracted using RIPA lysis buffer. Proteins were separated by 10% SDS-PAGE and blotted on PVDF membranes (Millipore, Billerica, MA, USA). After blocked with 5% skim milk, membranes were cultured with primary antibodies (Abcam, USA) at 4°C for one night. Then, membranes were cultured with the secondary antibody for 2 h. The membranes were visualized by ECL reagent (Beckman Colter, Brea, CA, USA). The relative densities of protein bands were analyzed by ImageJ.

2.10. ChIP assay

Cells were cross-linked with 1% formaldehyde. Next, lysis buffer was placed into the treated cells and chromatin was sheared to DNA fragments of 150–900 bp by sonication. After that, anti-p65 (Abcam) or anti-IgG was added into the sonicated mixtures for incubation. The precipitated complexes were rinsed and reverse cross-linked. After purification, the extracted DNA was subjected to amplification by RT-qPCR analysis.

2.11. Luciferase reporter assay

The p65 binding sites to Lcp1 promoter were inserted into the pGL3 luciferase reporter vector (Promega, Madison, WI, USA). Cells were subjected to co-transfection with pGL3-Lcp1 and si-p65 or si-NC for 48 h. Dual-Luciferase Reporter System (Promega) was applied to test the luciferase activity.

2.12. Statistical analysis

GraphPad Prism software (version 7.0, USA) was utilized to analyze the data. The measurement data were expressed as means ± SD from three individual repeats. Student's t-test was applied for comparison between two groups. The comparison among multiple groups was analyzed by the one-way ANOVA. $P<0.05$ indicated a statistically significant difference.

3. Results

3.1. Effects of TET on paw volume, gait score, and histological score of MIA mice

Through the utilizing of pubchem database (https://pubchem.ncbi.nlm.nih.gov/), we obtained the 2D and 3D structural formulas of TET and they were shown in Figures 1A, B. In order to explore the function of TET on GA process, we established the MIA mouse model and treated mice with different doses of TET (2 mg/kg, 4 mg/kg, and 8 mg/kg). Paw volume and gait score were commonly utilized for measuring the degree of joint swelling and the situation of joint dysfunction (Figures 1C, D). The results displayed that paw volume and gait score were significantly elevated in MIA mice in comparison to control mice. The treatment of TET notably reduced the two test indicators in a dose-dependent manner in comparison to MIA mice. COL is clinically used to treat gout, and here is used as a positive control. Obviously, COL can also reduce paw volume and gait score in MIA mice. Then, it was illustrated by HE staining that, compared with the control mice, there was obvious inflammatory cell infiltration, synovial hyperplasia and tissue necrosis in the ankle joint tissues of MIA mice. TET (4 mg/kg and 8 mg/kg) treatment significantly reduced this phenomenon, while 2 mg/kg of TET has little effect (Figure 1E). Thus, we confirmed that TET could alleviate GA in mice.

3.2. Effects of TET on macrophage repolarization markers in MIA mice

The effect of TET on inflammatory reaction and macrophage repolarization was further investigated. ELISA results indicated that IL-1β content in serum of MIA mice was significantly increased compared with control mice, while TET treatment (4 mg/kg, and 8 mg/kg) markedly reduced its content (Figure 2A). By contraries, IL-10 content reduced in serum of MIA mice was gradually recovered by TET treatment (4 mg/kg, and 8 mg/kg) (Figure 2B). Then, western blot was utilized to detect the levels of iNOS (M1 macrophage marker) and Arg-1 (M2 macrophage marker) in tissues. As a result, iNOS levels were increased in tissues of MIA group, while TET treatment reduced its levels. Arg-1 levels decreased in MIA mice were promoted by TET treatment (Figure 2C). Next, the expression of M1/M2-related genes was further detected. We found that TET declined IL-6, IL-1β, TNF-α, IL-12, and iNOS (M1-related genes) expression in tissues of MIA mice, but it elevated Mgl1, Mgl2, Pgc1-β, Arg-1, and IL-10 (M2-related genes) expression (Figures 2D, E). These outcomes suggested that TET can inhibit M1 macrophage repolarization and promote M2 macrophage repolarization in GA.

3.3. TET inhibits NF-κB-mediated Lcp1 expression in MIA mice

The NF-κB pathway is well-known as an inflammation-related pathway and has been repeatedly shown to be activated in GA [19, 20]. We found that NF-κB p65 activity was enhanced in tissues of MIA mice by using

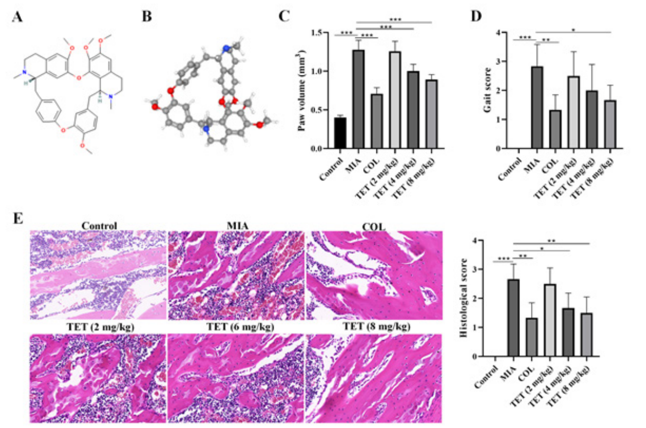

Fig. 1. Effects of TET on paw volume, gait score, and histological score of MIA mice. (A, B) The 2D and 3D chemical structural formula of TET was obtained from the PubChem database. (C, D) The paw volume and gait score of mice in the control group, the MIA group, the Col group, and the TET group were measured. (E) HE staining was utilized to evaluate the pathological change of ankle joint tissues of mice in different groups. *p<0.05, **p<0.01, ***p<0.001.

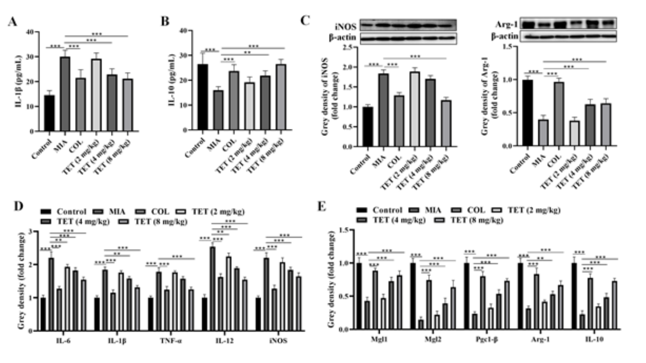

Fig. 2. Effects of TET on macrophage repolarization markers in MIA mice. (A, B) ELISA was utilized to detect the contents of IL-1β and IL-10 in serum of mice in the control group, the MIA group, the Col group, and the TET group. (C) Western blot of iNOS and Arg-1 levels in different groups. (D, E) Western blot of M1-related factors (IL-6, IL-1β, TNF-α, IL-12, and iNOS) and M2-related factors (Mgl1, Mgl2, Pgc1-β, Arg-1, and IL-10) in tissues. **p<0.01, ***p<0.001.

the commercial kit, however, TET treatment inhibited its activity (Figure 3A). To further explore the specific molecular mechanism, we screened the MSU-induced aberrant upregulated genes through the GSE199950 database and identified the downstream target genes of RELA (i.e. NF-κB p65) through the Cistrome Data Browser website (http://cistrome.org/db/). After the intersection of the genes found in both databases, only one gene was screened, Lcp1 (Figure 3B). By RT-qPCR, we found that Lcp1 was significantly upregulated in MIA mice, and its expression was decreased after TET treatment (Figure 3C). ChIP assay was used to detect the interaction between p65 and Lcp1. As a result, p65 can bind to the promoter region of Lcp1 in tissues and RAW264.7 cells (Figure 3D). Through the JASPAR website (https://jaspar.genereg.net/), we obtained the binding sites of p65 on the promoter region of Lcp1, and the schematic diagram is shown in Figure 3E. Then, the luciferase activity of the Lcp1 promoter was observed to decline when knocking down p65 in HEK-293T and RAW264.7 cells, further confirming the combination of Lcp1 promoter and p65 (Figure 3F). We also observed that the expression level of Lcp1 was markedly suppressed upon knockdown of p65 (Figure 3G). These results confirm that p65 can transcriptionally activate the expression of Lcp1. We therefore concluded that TET inhibited NF-κB-mediated Lcp1 expression in MIA mice.

3.4. Effects of Lcp1 silencing on joint swelling and joint dysfunction in MIA mice

We purchased the Lcp1 KO mice to further detect the effect of Lcp1 silencing on joint swelling and joint dysfunction of MIA mice. We discovered that, compared with the control MIA mice, Lcp1 KO MIA mice showed less paw volume and lower gait score, suggesting Lcp1 silencing notably alleviated joint swelling and joint dysfunction in MIA mice. TET treatment further enhanced the effect of Lcp1 silencing on joint swelling and joint function (Figures 4A, B). Furthermore, HE staining showed that compared with the significant inflammatory infiltration, synovial hyperplasia and necrosis in the tissues of MIA group, we observed that these pathological changes were significantly alleviated in MIA+Lcp1 KO group, and the treatment effect was more obvious after TET administration (Figure 4C). The same trend appeared in the histological score (Figure 4D). Thus, we confirmed that TET improved GA by regulating Lcp1 expression.

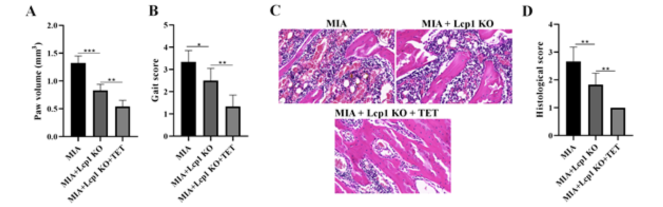

Fig. 4. Effects of Lcp1 silencing on joint swelling and joint dysfunction in MIA mice. (A, B) The paw volume and gait score of mice in the MIA group, the MIA+Lcp1 KO group, and the MIA+Lcp1 KO+TET group were measured. (C, D) HE staining was utilized to evaluate the pathological change of ankle joint tissues of mice in different groups. *p<0.05, **p<0.01, ***p<0.001.

3.5. Effects of Lcp1 silencing on macrophage repolarization markers in MIA mice

ELISA was implemented to test the impact of Lcp1 depletion on inflammatory factors. The results illustrated that Lcp1 silencing reduced the IL-1β content and increased IL-10 content and TET treatment further enhanced the effect of Lcp1 silencing (Figures 5A, B). Then we observed that in comparison of control MIA mice, iNOS, IL-6, IL-1β, TNF-α, and IL-12 levels in Lcp1 KO MIA mice were notably decreased, while Arg-1, Mgl1, Mgl2, Pgc1-β, and IL-10 levels were increased. Furthermore, TET administration further strengthened the function of Lcp1 knockdown (Figures 5C-E). In short, these results proved that TET promoted M2 macrophage repolarization by regulating Lcp1 in GA.

4. Discussion

GA is an inflammatory disease caused by the deposition of MSU crystals into joints [1]. In recent years, with the in-depth study of the pathogenesis of GA, a variety of traditional Chinese medicines have been proven to have a significant improvement effect on the development of GA, such as Simiao Decoction [21], Resveratrol [22], and Si-Miao-San [10]. As a bisbenzylisoquinoline alkaloid, TET is widely used in different diseases due to its anti-inflammatory, analgesic and other effects [14]. For instance, TET regulates Rheb-mTOR pathway-mediated autophagy to protect against pulmonary fibrosis [23]. TET relieves silicosis via suppressing NLRP3 inflammasome in lung macrophages [24]. TET alleviates ischemia/reperfusion-induced neuronal damage in the subacute phase [25]. Furthermore, studies have confirmed that TET also exerts protective effects on arthritis by alleviating neutrophil activities [17], inactivating NF-κB [16], and inhibiting inhibiting osteoclastogenesis [26]. In this study, the GA mouse model was established by MSU injection into ankle joint. We found that 4 mg/kg and 8 mg/kg of TET significantly alleviated joint swelling and dysfunction. Histopathological analysis showed that TET alleviated inflammatory cell infiltration and tissue necrosis at the joint injury site, and its effect was equivalent to that of colchicine. Therefore,

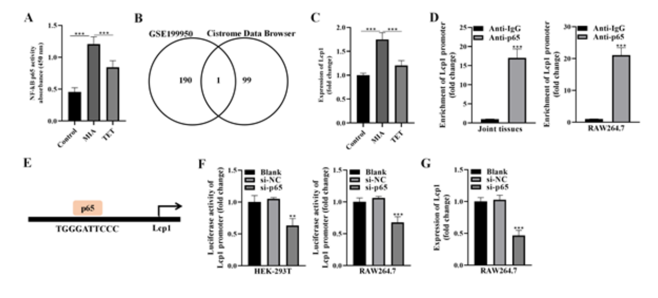

Fig. 3. TET inhibits NF-κB-mediated Lcp1 expression in MIA mice. (A) The activity of NF-κB p65 in tissues of control group, the MIA group, and the TET group was tested by the commercial kit. (B) GSE199950 dataset and Cistrome Data Browser database were utilized to predict the genes induced by MSU and the downstream of the NF-κB pathway. (C) RT-qPCR result of Lcp1 expression in tissues of the control group, the MIA group, and the TET group. (D) ChIP assay was utilized to determine the interaction of p65 and Lcp1 in tissues and cells. (E) The binding sites of p65 on Lcp1 promoter were predicted by JASPAR database. (F) Luciferase reporter assay was applied to determine the combination of p65 and Lcp1 promoter. (G) RT-qPCR result of Lcp1 expression in cells when p65 was silenced. **p<0.01, ***p<0.001.

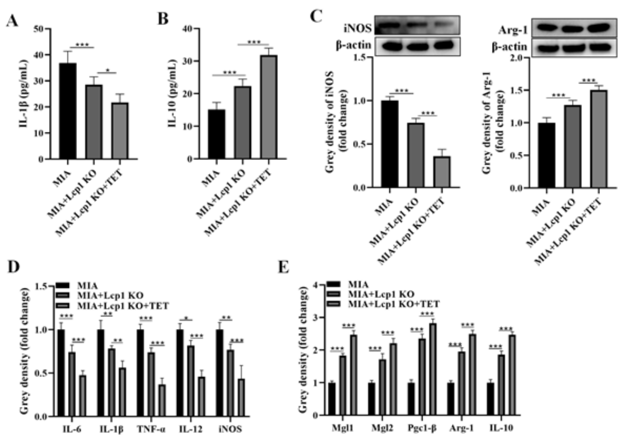

Fig. 5. Effects of Lcp1 silencing on macrophage repolarization markers in MIA mice. (A, B) ELISA was utilized to detect the contents of IL-1β and IL-10 in serum of mice in the MIA group, the MIA+Lcp1 KO group, and the MIA+Lcp1 KO+TET group. (C) Western blot of iNOS and Arg-1 levels in different groups. (D, E) Western blot of M1-related factors (IL-6, IL-1β, TNF-α, IL-12, and iNOS) and M2-related factors (Mgl1, Mgl2, Pgc1-β, Arg-1, and IL-10) in tissues. *p<0.05, **p<0.01, ***p<0.001.

we believe that TET can alleviate the process of GA.

After MSU is recognized and activated in the joint tissues, a large number of pro-inflammatory mediators, including IL-1β, IL-6, TNF-α and chemokines, are transcribed and released, resulting in an acute inflammatory response [27, 28]. Subsequently, neutrophils enter the joint or peri-articular tissues and engulf the deposited MSU crystals, releasing inflammatory cytokines and mononuclear macrophage to amplify and maintain joint inflammation, resulting in further joint swelling [27, 28]. M1 macrophages can secrete lots of pro-inflammatory cytokines. In contrast, M2 macrophages mainly produce anti-inflammatory factors, such as IL-10, TGF-β, and Arg1 [29]. The core of pro-inflammatory switch is the expression of iNOS, which produces a large amount of nitric oxide [30]. Similarly, a key biomarker of M2 activation is the increase of Arg-1 level, which can decompose arginine and inhibit the production of nitric oxide [31, 32]. This study found that M1-related pro-inflammatory factors (IL-6, IL-1β, TNF-α, IL-12, and iNOS) were upregulated in MSU-induced mice, while TET treatment inhibited their expression. On the contrary, the stimulation of MSU declined the expression of M2-related anti-inflammatory factors (Mgl1, Mgl2, Pgc1-β, Arg-1, and IL-10), but TET treatment reversed this situation. Therefore, we believe that TET can alleviate GA development by inducing M2 macrophage polarization.

NF-κB is a vital transcriptional factor of M1 macrophage polarization, which modulates the transcription of many inflammatory genes related to M1 macrophage [19, 33]. Furthermore, MSU crystal has been proven to activate NF-κB through TLR-mediated MyD88 complex [34]. It is reported that NF-κB levels in GA patients were significantly higher than in healthy ones [35]. Isovitexin is confirmed to suppress inflammatory reactions to relieve GA in rats via the inhibition of TLR4/MyD88/NF-κB pathway [36]. In this study, we discovered that NF-κB p65 activity was upregulated in MUS-induced mice, while TET reduced its activity. Lcp1 is a cytosolic actin-binding protein. Studies have revealed that Lcp1 is mainly expressed in the stroma of osteoarthritis synovium, and its expression is significantly increased under inflammation [37]. It is reported that Lcp1 inactivation can reduce the occurrence and development of anti-type II collagen-induced arthritis in mice and has a protective effect on bone erosion [38]. Through bioinformatics tools, Lcp1 was found to be both the upregulated gene of MSU and the downstream gene of NF-κB pathway. Our study found that TET treatment inhibited the upregulated expression of Lcp1 in the joint tissue of model mice. We further confirmed that p65 can bind to Lcp1 promoter, and p65 activates Lcp1 expression. Compared with conventional model mice, Lcp1 KO mice showed less joint injury and better behavioral function after MSU induction. Interestingly, TET treatment further promoted the repair of injured joints in Lcp1 KO model mice. A study has confirmed that Lcp1 depletion may cause altered cell skeletons thus leading to cell shape alterations, and resulting in macrophage polarization change from the M1 to M2 phenotype [39]. Similarly, we observed that the knockdown of Lcp1 inhibited the MSU-induced inflammatory response and activated macrophage M2 polarization, and TET administration enhanced its effect.

Overall, this study confirmed that TET can alleviate inflammation and promote macrophage M2 polarization to suppress GA process by NF-κB-mediated Lcp1. These discoveries suggest TET may become a new and effective agent for GA.

Informed Consent

The authors report no conflict of interest.

Availability of data and material

We declared that we embedded all data in the manuscript.

Authors' contributions

FL and SR conducted the experiments and wrote the paper; LY analyzed and organized the data; XX and HF conceived, designed the study and revised the manuscript.

Funding

This work was supported by the TCM Science and Technology Program of Zhejiang Province (No. 2023ZF192) and Zhoushan Medical and Health Science and Technology Project of Zhejiang Province (No. 2023ZD01), and Zhoushan Medical and Health Research Special Funds.

Acknowledgement

We thanked Zhoushan Hospital of Zhejiang Province, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine and Zhoushan Hospital of Traditional Chinese Medicine for approving our study.

References

1. Tausche AK, Aringer M (2016) [Gouty arthritis]. Z Rheumatol 75(9):885–898. doi: 10.1007/s00393-016-0206-z
2. Desai J, Steiger S, Anders HJ (2017) Molecular pathophysiology of gout. Trends Mol Med 23(8):756–768. doi: 10.1016/j.molmed.2017.06.005
3. Keyßer G (2020) [Gout arthritis: Pathogenesis, diagnostics and treatment]. Dtsch Med Wochenschr 145(14):991–1005. doi: 10.1055/a-1036-8348
4. Keller SF, Mandell BF (2021) Management and cure of gouty

arthritis. Med Clin North Am 105(2):297–310. doi: 10.1016/j.mcna.2020.09.013
5. Keller SF, Mandell BF (2022) Management and cure of gouty arthritis. Rheum Dis Clin North Am 48(2):479–492. doi: 10.1016/j.rdc.2022.03.001
6. Liang H, Deng P, Ma YF, Wu Y, Ma ZH, Zhang W, et al (2021) Advances in experimental and clinical research of the gouty arthritis treatment with traditional chinese medicine. Evid Based Complement Alternat Med 2021:8698232. doi: 10.1155/2021/8698232
7. Wynn TA, Vannella KM (2016) Macrophages in tissue repair, regeneration, and fibrosis. Immunity 44(3):450–462. doi: 10.1016/j.immuni.2016.02.015
8. Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, et al (2018) Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol 233(9):6425–6440. doi: 10.1002/jcp.26429
9. Gao J, Liang Y, Wang L (2022) Shaping polarization of tumor-associated macrophages in cancer immunotherapy. Front Immunol 13:888713. doi: 10.3389/fimmu.2022.888713
10. Cao L, Zhao T, Xue Y, Xue L, Chen Y, Quan F, et al (2021) The anti-inflammatory and uric acid lowering effects of Si-Miao-San on gout. Front Immunol 12:777522. doi: 10.3389/fimmu.2021.777522
11. Mei J, Zhou F, Qiao H, Li H, Tang T (2019) Nerve modulation therapy in gouty arthritis: targeting increased sFRP2 expression in dorsal root ganglion regulates macrophage polarization and alleviates endothelial damage. Theranostics 9(13):3707–3722. doi: 10.7150/thno.33908
12. Zhou F, Mei J, Han X, Li H, Yang S, Wang M, et al (2019) Kinsenoside attenuates osteoarthritis by repolarizing macrophages through inactivating NF-κB/MAPK signaling and protecting chondrocytes. Acta Pharm Sin B 9(5):973–985. doi: 10.1016/j.apsb.2019.01.015
13. Yang J, Chen G, Guo TW, Qin WY, Jia P (2021) Simiao Wan attenuates monosodium urate crystal-induced arthritis in rats through contributing to macrophage M2 polarization. J Ethnopharmacol 275:114123. doi: 10.1016/j.jep.2021.114123
14. Bhagya N, Chandrashekar KR (2016) Tetrandrine--A molecule of wide bioactivity. Phytochemistry 125:5–13. doi: 10.1016/j.phytochem.2016.02.005
15. N B, K RC (2018) Tetrandrine and cancer - An overview on the molecular approach. Biomed Pharmacother 97:624–632. doi: 10.1016/j.biopha.2017.10.116
16. Gao LN, Feng QS, Zhang XF, Wang QS, Cui YL (2016) Tetrandrine suppresses articular inflammatory response by inhibiting pro-inflammatory factors via NF-κB inactivation. J Orthop Res 34(9):1557–1568. doi: 10.1002/jor.23155
17. Lu Q, Jiang H, Zhu Q, Xu J, Cai Y, Huo G, et al (2022) Tetrandrine ameliorates rheumatoid arthritis in mice by alleviating neutrophil activities. Evid Based Complement Alternat Med 2022:8589121. doi: 10.1155/2022/8589121
18. Lv Q, Zhu XY, Xia YF, Dai Y, Wei ZF (2015) Tetrandrine inhibits migration and invasion of rheumatoid arthritis fibroblast-like synoviocytes through down-regulating the expressions of Rac1, Cdc42, and RhoA GTPases and activation of the PI3K/Akt and JNK signaling pathways. Chin J Nat Med 13(11):831–841. doi: 10.1016/s1875-5364(15)30087-x
19. Lawrence T (2009) The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol 1(6):a001651. doi: 10.1101/cshperspect.a001651
20. Ouyang X, Li NZ, Guo MX, Zhang MM, Cheng J, Yi LT, Zhu JX (2021) Active flavonoids from lagotis brachystachya attenuate monosodium urate-induced gouty arthritis via inhibiting TLR4/MyD88/NF-κB pathway and NLRP3 expression. Front Pharmacol 12:760331. doi: 10.3389/fphar.2021.760331
21. Lin X, Shao T, Huang L, Wen X, Wang M, Wen C, He Z (2020) Simiao decoction alleviates gouty arthritis by modulating proinflammatory cytokines and the gut ecosystem. Front Pharmacol 11:955. doi: 10.3389/fphar.2020.00955
22. Fan W, Chen S, Wu X, Zhu J, Li J (2021) Resveratrol relieves gouty arthritis by promoting mitophagy to inhibit activation of NLRP3 inflammasomes. J Inflamm Res 14:3523–3536. doi: 10.2147/jir.S320912
23. Liu Y, Zhong W, Zhang J, Chen W, Lu Y, Qiao Y, et al (2021) Tetrandrine modulates Rheb-mTOR signaling-mediated selective autophagy and protects pulmonary fibrosis. Front Pharmacol 12:739220. doi: 10.3389/fphar.2021.739220
24. Song MY, Wang JX, Sun YL, Han ZF, Zhou YT, Liu Y, et al (2022) Tetrandrine alleviates silicosis by inhibiting canonical and non-canonical NLRP3 inflammasome activation in lung macrophages. Acta Pharmacol Sin 43(5):1274–1284. doi: 10.1038/s41401-021-00693-6
25. Wang Y, Cai X, Wu Z, Tang L, Lu L, Xu Y, Bao X (2021) Tetrandrine attenuates ischemia/reperfusion-induced neuronal damage in the subacute phase. Mol Med Rep 23(4):297. doi: 10.3892/mmr.2021.11936
26. Jia Y, Tao Y, Lv C, Xia Y, Wei Z, Dai Y (2019) Tetrandrine enhances the ubiquitination and degradation of Syk through an AhR-c-src-c-Cbl pathway and consequently inhibits osteoclastogenesis and bone destruction in arthritis. Cell Death Dis 10(2):38. doi: 10.1038/s41419-018-1286-2
27. Meng Q, Meng W, Bian H, Zheng F, Gu H, Zuo R, et al (2021) Total glucosides of paeony protects THP-1 macrophages against monosodium urate-induced inflammation via MALAT1/miR-876-5p/NLRP3 signaling cascade in gouty arthritis. Biomed Pharmacother 138:111413. doi: 10.1016/j.biopha.2021.111413
28. Keenan RT (2020) The biology of urate. Semin Arthritis Rheum 50(3):S2–S10. doi: 10.1016/j.semarthrit.2020.04.007
29. Yunna C, Mengru H, Lei W, Weidong C (2020) Macrophage M1/M2 polarization. Eur J Pharmacol 877:173090. doi: 10.1016/j.ejphar.2020.173090
30. Orecchioni M, Ghosheh Y, Pramod AB, Ley K (2019) Macrophage polarization: different gene signatures in M1(LPS+) vs. classically and M2(LPS-) vs. alternatively activated macrophages. Front Immunol 10:1084. doi: 10.3389/fimmu.2019.01084
31. Gong M, Zhuo X, Ma A (2017) STAT6 upregulation promotes M2 macrophage polarization to suppress atherosclerosis. Med Sci Monit Basic Res 23:240–249. doi: 10.12659/msmbr.904014
32. Kuruppu S, Rajapakse NW, Dunstan RA, Smith AI (2014) Nitric oxide inhibits the production of soluble endothelin converting enzyme-1. Mol Cell Biochem 396(1-2):49–54. doi: 10.1007/s11010-014-2141-0
33. Liu L, Guo H, Song A, Huang J, Zhang Y, Jin S, et al (2020) Progranulin inhibits LPS-induced macrophage M1 polarization via NF-κB and MAPK pathways. BMC Immunol 21(1):32. doi: 10.1186/s12865-020-00355-y
34. Shen R, Ma L, Zheng Y (2020) Anti-inflammatory effects of luteolin on acute gouty arthritis rats via TLR/MyD88/NF-κB pathway. Zhong Nan Da Xue Xue Bao Yi Xue Ban 45(2):115–122. doi: 10.11817/j.issn.1672-7347.2020.190566
35. Cao Y (2021) Icariin alleviates MSU-induced rat GA models through NF-κB/NALP3 pathway. Cell Biochem Funct 39(3):357–366. doi: 10.1002/cbf.3598
36. Sun X, Li P, Qu X, Liu W (2021) Isovitexin alleviates acute gouty arthritis in rats by inhibiting inflammation via the TLR4/MyD88/NF-κB pathway. Pharm Biol 59(1):1326–1333. doi: 10.1080/13880209.2021.1979595
37. de Seny D, Baiwir D, Bianchi E, Cobraiville G, Deroyer C, Poulet

C, et al (2021) New proteins contributing to immune cell infiltration and pannus formation of synovial membrane from arthritis diseases. Int J Mol Sci 23(1):434. doi: 10.3390/ijms23010434
38. Sehnert B, Cavcic A, Böhm B, Kalden JR, Nandakumar KS, Holmdahl R, Burkhardt H (2004) Antileukoproteinase: modulation of neutrophil function and therapeutic effects on anti-type II collagen antibody-induced arthritis. Arthritis Rheum 50(7):2347–2359. doi: 10.1002/art.20339
39. Wang Y, Luo Y, Yao Y, Ji Y, Feng L, Du F, et al (2020) Silencing the lncRNA Maclpil in pro-inflammatory macrophages attenuates acute experimental ischemic stroke via LCP1 in mice. J Cereb Blood Flow Metab 40(4):747–759. doi: 10.1177/0271678x19836118