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Resveratrol alleviates MSU-induced gouty arthritis in rats through inhibition of HIF-1α- and NLRP3-derived IL-1β secretion in macrophages

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ABSTRACT

Gouty arthritis is an acute and chronic joint inflammatory joint disease characterized by the deposition of monosodium urate (MSU) crystals in joints and periarticular tissues. Resveratrol (3, 5, 4-trihydroxy-trans-stilbene, RV), a natural polyphenolic compound, has anti-inflammatory and antioxidant properties. The purpose of this study was to investigate the effect of resveratrol on rats with gouty arthritis and its molecular mechanism. THP-1-derived macrophages were induced by lipopolysaccharide (LPS) and MSU to create an in vitro gout cell inflammation model, and rats were injected with MSU crystals into the right ankle joint for an in vivo acute gouty arthritis model. We investigated the anti-inflammatory properties of resveratrol using these in vitro and in vitro models. Our findings suggested that resveratrol effectively reduced ankle swelling and synovial inflammation in a dose-dependent manner in rats with acute gouty arthritis, with almost the same effect as colchicine treatment. In MSU-treated THP-1-derived macrophages, resveratrol inhibited NLRP3 inflammasome activation and IL-1β secretion. Furthermore, resveratrol and the HIF-1α inhibitor PX478 both inhibited the expression of the NLRP3 inflammasome, IL-1β, and HIF-1α. This study demonstrated that resveratrol significantly improved the symptoms of acute gouty arthritis and its potential mechanism may be IL-1β reduction via HIF-1α modulation and inhibition of NLRP3 inflammasome activation. Our study might offer a novel sight for the treatment of gouty arthritis.

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Introduction

Gouty arthritis is an autoinflammatory disorder characterized by monosodium urate (MSU) crystal deposition in articular and periarticular tissues, which is related to hyperuricemia (1). Gout clinically manifests as recurrent attacks of joint pain, redness, and dysfunction. In severe cases, it can lead to joint deformity and even kidney injury (2). The prevalence of gout ranges from a prevalence of <1% to 6. 8% and an incidence of 0. 58-2. 89 per 1, 000 person-years in different regions of the world (3). The initiating mechanism of an acute gout attack is the stimulation of macrophages by MSU crystals to activate the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, an inflammatory protein complex composed of the innate immune receptor NLRP3, linker protein apoptosis-associated speck-like protein (ASC) and protease caspase-1, resulting in the maturation and release of interleukin-1β (IL-1β) (4). The secretion of IL-1β requires two stages: the first stage (priming) is mediated by Toll-like receptor (TLR) activation and the NF-κB pathway, a signaling cascade that induces the activation of NLRP3, and the second signal (activation) promotes NLRP3 inflammasome assembly and ASC recruitment into the inflammasome. Activated caspase-1 promotes pro-IL-1β proteolysis into biologically active IL-1β, causing the inflammatory process of gout (2, 5, 6).

Macrophages are key effector cells in acute gout attacks. TLR ligands activate macrophages, converting their core metabolism from oxidative phosphorylation to glycolysis, and glycolysis is closely related to inflammatory reactions (7). Hypoxia-inducible factor 1 α (HIF-1α) is a key regulator of glycolysis that induces the expression of glycolytic enzymes that promote continuous energy generation. HIF-1α plays a significant role in the immune/inflammatory response (8). Succinate, the Krebs cycle metabolite, is an inflammatory signal that induces IL-1β expression by regulating HIF-1α (7). Succinate is one of the most common immunometabolites in macrophages, when an acute gout attack starts, macrophages activate TLR4, which blocks the Krebs cycle, restricting succinate oxidation and resulting in succinate accumulation (9). Traditionally, HIF-1α is regulated by prolyl hydroxylases (PHDs), which induce proteasomal degradation of HIF-1α by facilitating hydroxylation (10). However, elevated succinate levels inhibit PHDs and activate HIF-1α transcriptional activity, which results in the accumulation of HIF-1α in macrophages under inflammatory conditions, enhancing the production of IL-1β (8, 9). Considering this accumulation of HIF-1α in macrophages during episodes of an acute gout attack, HIF-1α is a potential therapeutic avenue for acute gouty arthritis.

Resveratrol (3, 5, 4-trihydroxy-trans-stilbene, RV), a natural phytoalexin and a polyphenolic compound, is hi-

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ghly concentrated in grapes, *Polygonum cuspidatum* and other plants (11). The accumulated evidence on resveratrol has confirmed its potential applications in the prevention and treatment of inflammation-related diseases (12-14). Moreover, resveratrol can be a useful medicine for treating NLRP3-associated diseases (15). Therefore, we believe that the antioxidant resveratrol can help prevent acute gouty arthritis. Furthermore, resveratrol has been shown to reduce the MSU-induced inflammatory response by inhibiting TAK1 activity (16), and it alleviates gouty arthritis by promoting mitophagy to inhibit NLRP3 activation (17). Resveratrol is beneficial in the treatment of inflammatory diseases and tumors (3). Furthermore, resveratrol inhibits cancer cell glucose uptake by regulating HIF-1α activation (18). However, the underlying mechanism of resveratrol mediation of the NLRP3 inflammasome and the expression of HIF-1α in gout remains largely unclear.

We uncovered the role of the NLRP3 inflammasome and HIF-1α in the pathogenesis of MSU-induced acute gouty arthritis models in vitro and in vivo, providing insight into the effect of resveratrol in acute gouty arthritis.

Materials and Methods

Animals

Specific pathogen-free (SPF) male Sprague Dawley (SD) rats, 200–220 g, 6–8 weeks old, were provided by Chengdu Enswell Biotechnology Co. Ltd. (Chengdu, China). The animals were reared in a standard environment of temperature (23 ± 1 °C) and relative humidity (55 ± 5%). All experimental procedures were approved by the Ethics Committee of the Western Theater Command General Hospital, the authorization number is 2022EC3—ky 061.

Cell culture and Treatment

Human THP-1 cells were supplied by Wuhan Pu-nuosai Life Technology Co. Ltd. (Wuhan, China). Routine culture of THP-1 cells was performed in RPMI 1640 (Gibco) containing 10% fetal bovine serum (FBS, Gibco), 1% penicillin/streptomycin, and 0. 05 mM β-mercaptoethanol at 37 °C and 5% CO_2. THP-1 cells in the logarithmic growth phase were induced to differentiate into macrophages using 100 nM horbol-12-myristate-13-acetate (PMA, Abcam) for 24 h (19, 20).

Cell viability assay

The Cell Counting Kit 8 (CCK-8, Biosharp Life Sciences) was used to examine the effect of resveratrol on the viability of THP-1-derived macrophages. We seeded THP-1 cells (1×10^6 cells/ml) in a 96-well plate, stimulated them with 100 nM PMA overnight (37 °C, 5% CO_2), removed the culture medium and washed the cells three times with PBS. Resveratrol (Sigma–Aldrich) was added at final concentrations of 0, 5, 10, 50, 100, 200, 300, 400 and 500 μM for 24 h. After the resveratrol treatment, we added 100 μl RPMI-1640 complete medium-diluted CCK-8 reagent to each well and then measured the optical density (OD) at 450 nm of each well using a microplate reader after 1 h of incubation at 37 °C.

Similarly, in 96-well plates, THP-1 cells were seeded (1×10^6 cells/ml) and stimulated with 100 nM PMA overnight. After pretreatment with 100 ng/ml LPS (Sigma–Aldrich) for 3 h, cells were incubated with resveratrol (0, 5, 10, 20 50, 70, 100 μM) and 200 μg/ml MSU (InvivoGen) for 24 h. Cell viability was then determined as described above. Cell viability was calculated using the formula:

$$cell\ viability(\%) = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\%$$

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In vitro MSU-Induced Acute Gouty Arthritis Model

THP-1 cells (1×10^6 cells/mL) were seeded in a 6-well plate and stimulated with 100 nM PMA overnight. After the removal of the culture media, the cells were washed three times with PBS. Two groups were treated separately after 3 hours of LPS (100 ng/ml) addition. Group I: cells were incubated with resveratrol (0, 20, 50, 100 μM) and MSU (200 μg/ml) for 24 h. Group II: The cells were stimulated with MSU (200 μg/ml), then co-cultured with resveratrol and PX478 (MCE) for 24 h, respectively.

Creating an MSU-Induced Acute Gouty Arthritis Rat Model

The model was constructed based on pre-experiment and previous studies (21, 22). 36 rats were randomly assigned to one of six groups: the control group (control), model group (MSU), colchicine group (Col), low-dose resveratrol group (res L), middle-dose resveratrol group (res M) and high-dose resveratrol group (res H). The medicine was administered intragastrically for 7 days, with rats in the drug treatment groups receiving resveratrol (10, 20 or 50 mg/kg/day) or colchicine (0. 3 mg/kg/day). Both resveratrol and colchicine were dissolved using 2 ml of PBS. Rats in the control and model groups were given the same volume of PBS. On day 5, following dosing, all groups except the control group were injected with 0. 1 ml of MSU suspension (50 mg/ml) into the right ankle joint cavity, while the control group received an equal volume of phosphate-buffered saline (PBS). To assess ankle joint swelling, the ankle circumference of each rat was measured at the beginning and end of the experiment. The ankle joints were removed to perform a pathomorphological study. The ankle swelling index was calculated using the following formula:

$$Swelling\ index(\%) = \frac{V_{after\ injection} - V_{before\ injection}}{V_{before\ injection}}$$

Histopathological Analysis

The degree of inflammatory cell infiltration in rat synovial tissues was assessed using hematoxylin-eosin (HE) staining. The ankle tissues were fixed with 4% paraformaldehyde solution, decalcified with 10% ethylenediamine tetraacetic acid (EDTA), embedded in paraffin and sectioned into 4 μm thickness. Followed by standard histological protocols, the samples were stained with hematoxylin for 5 min. After washing, eosin was added for incubation for about 2 min. The images were captured using an Olympus VS200 slide scanner light microscope with 200× total magnification.

IL-1β and HIF-1α Detection by ELISA

Centrifugation at 1500 rpm/min for 20 minutes at 4 °C was used to collect cell culture supernatants from MSU-treated THP-1-derived macrophages. IL-1β and HIF-1α were measured in cell culture supernatants using human IL-1β and HIF-1α enzyme-linked immunosorbent assay (ELISA) kits (Abcam, USA) according to the manufacturer's protocols.

Table 1. Primer sequences.

Gene		Primer sequence
IL-1β	Forward	5'-TGCTCAAGTGTCTGAAGCAG-3'
	Reverse	5'-TGGTGGTCGGAGATTCGTAG-3'
HIF-1α	Forward	5'-GAACGTCGAAAAGAAAAGTCTCG-3'
	Reverse	5'-CCTTATCAAGATGCGAACTCACA-3'
β-actin	Forward	5'-GAGCTACGAGCTGCCTGACG-3'
	Reverse	5'-GTAGTTTCGTGGATGCCACAG-3'

Western Blotting Analysis

The protein samples from each group were loaded onto 10% separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene fluoride (PVDF) membranes. After being blocked, The PVDF membranes were incubated overnight at 4 °C with the corresponding primary antibodies for NLRP3 (1:1000; Abcam), HIF-1α (1:1000; Abcam), ASC (1:1000; Abcam), Caspase-1 (1:1000; ABclonal), IL-1β (1:1000; Cell Signaling Technology), β-actin antibody (1:5000; Proteintech). Then the membranes were incubated with secondary antibodies (1:2000, Abcam) for 1 h. The enhanced chemiluminescent (ECL) visualization system was used to visualize the protein signals, and the blots were analyzed for optical density using ImageJ.

RNA preparation and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from the THP-1 cell groups using TRIzol reagent (Invitrogen, USA). RT–PCR was performed employing a One-Step TB Green™ PrimeScript™ RT–PCR Kit II (Takara, Japan). The experiment was performed with Thermal Cycler Dice Real-time system series amplifiers. The first step was a reverse-transcription reaction (held at 42 °C for 5 min and 95 °C for 10 sec), and the second step was PCR (40 times at 95 °C for 5 sec and 60 °C for 30 sec). The last step was dissociation. Relative gene expression was calculated using the $2^{-(\Delta\Delta Ct)}$ method. The primer sequences used were shown in Table 1.

Immunofluorescence Detection

THP-1 cells treated as described above were collected, fixed with 4% paraformaldehyde, and permeabilized with 0. 1% Triton X-100 at room temperature. Then, the cells were blocked with 5% goat serum for 30 min and incubated with primary HIF-1α (1:100, Abcam) or ASC antibody (1:100, Abcam) at 4 °C overnight. The cells were then washed with PBS and labeled with secondary antibodies (1:200, Abcam) at room temperature for 1 h, and 4', 6-diamidino-2-phenylindole (DAPI) was used to stain the nuclei. The cells were analyzed using a fluorescence microscope (Nikon) with 10× objectives, and the fluorescence intensity was quantified using Image-Pro Plus software. ASC specks (%) = ASC specks/total cell numbers.

Statistics

Each experiment was carried out at least three times. The data were analyzed using GraphPad Prism 8. 0 (GraphPad Software, La Jolla, CA, USA). All data are represented as the mean ± SEM. Comparisons between multiple groups were performed using ANOVA followed by Tukey's test. $P < 0.05$ indicates a statistically significant difference.

Results

Effect of resveratrol on THP-1 derived macrophage viability.

THP-1-derived macrophages were treated with resveratrol at various concentrations, and cell viability was measured using CCK-8. The results were shown in Figure 1A. Resveratrol had no impact on viability in the concentration range of 0-100 μM, but there was a significant decrease in cell viability when the concentration was above 100 μM. Hence, in the following experiments, the concentration of resveratrol used was 0-100 μM.

The protective effects of resveratrol with various concentrations on THP-1 cells stimulated by LPS and MSU were further investigated. MSU crystal stimulation significantly decreased THP-1 cell viability, as shown in Figure 1B, but resveratrol administration (0-100 μM) increased the viability of THP-1 cells, and there were no significant differences between the concentrations. Therefore, 20, 50 and 100 μM were used as intervention concentrations in the following experiments.

Resveratrol alleviated the inflammatory response in MSU-treated THP-1-derived macrophages

To examine whether resveratrol treatment had suppressive effects on inflammation, LPS/MSU-treated THP-1 cells were cotreated with resveratrol (20, 50, 100 μM) for 24 h. Secreted cytokines in the supernatants of treated THP-1-derived macrophages were detected by ELISA. As shown in Figure 1C, LPS induction and MSU stimulation significantly increased IL-1β secretion, but IL-1β secretion was decreased in a dose-dependent manner after resveratrol treatment. The secretion of IL-1β was the lowest at 100 μM resveratrol. The results of both RT-PCR for IL-1β mRNA and Western blot analysis for IL-1β protein expression were in agreement with the ELISA results (Figure 1D, 1E).

Resveratrol inhibited NLRP3 inflammasome activation in MSU-treated THP-1-derived macrophages

Since the NLRP3 inflammasome regulates inflammation by inducing IL-1β maturation and secretion, we investigated the role of NLRP3 in the effects of resveratrol on inflammation in MSU-treated THP-1-derived macrophages. As illustrated in Figure 1F, Western blot indicated that resveratrol inhibited the protein expression of NLRP3, ASC, and Caspase-1, but this effect was not dose-dependent.

HIF-1α was overexpressed in MSU-treated THP-1-derived macrophages, but resveratrol inhibited HIF-1α expression

THP-1-derived macrophages were induced with LPS for 3 h and then cocultured with MSU and varying concentrations of resveratrol for 24 h to investigate the effect of resveratrol on HIF-1α. RT- PCR was used to detect HIF-1α mRNA, as shown in Figure 1G, LPS induction and MSU stimulation significantly increased HIF-1α secretion, while HIF-1α secretion was decreased after resveratrol treatment, but not in a dose-dependent manner. ELISA and Western blotting yielded the same results (Figure 1H, 1I).

Resveratrol reduced the release of IL-1β by inhibiting HIF-1α activation and thereby suppressing inflammatory responses

It has been reported that HIF-1α regulates the production and release of IL-1β, and resveratrol can inhibit HIF-1α expression (7, 18). Therefore, we hypothesized that resveratrol could confer protective effects in MSU-treated THP-1-derived macrophages by suppressing HIF-1α expression. LPS/MSU-treated THP-1-derived macrophages were cotreated for 24 h with 100 μM resveratrol or HIF-1α inhibitor (PX478, 50 μM). Resveratrol reduced the protein expression of NLRP3, ASC, and caspase-1, as measured by western blot, and PX478 produced nearly identical results (Figure 2A). NLRP3 inflammasome activation promotes ASC oligomerization to form ASC specks, thereby recruiting and activating Caspase-1 (23). ASC oligomerization is a commonly used indicator of inflammasome activation (24). Therefore, we examined whether resveratrol affects ASC oligomerization. The results showed that ASC specks were significantly reduced in the resveratrol intervention group compared with the LPS/MSU-treated group. The same results as resveratrol treatment were obtained using PX478 (Figure 2B, 2C).

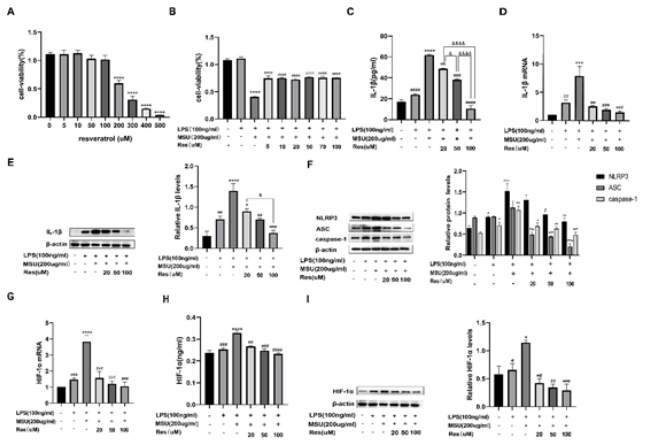

Figure 1. Resveratrol's effect on MSU-treated THP-1-derived macrophages. (A)THP-1-derived macrophages were treated for 24 hours with various concentrations of resveratrol (0-500 μM), and cell viability was determined using CCK-8 kits. (B) THP-1 cells were induced with PMA (100 nM) overnight and then treated for 24 hours with resveratrol (0, 5, 10, 20, 50, 70, and 100 μM) and MSU (200 μg/ml) after being pretreated with LPS (100 ng/ml) for 3 hours. Cell viability was assessed by CCK-8 assay. (C-I) THP-1 cells were induced with PMA (100 nM) overnight and then treated with resveratrol (20, 50, and 100 μM) and MSU (200 μg/ml) for 24 hours after being pretreated with LPS (100 ng/ml) for 3 hours. secreted IL-1β was measured in supernatants by ELISA (C), IL-1β mRNA was measured by RT–PCR (D), IL-1β protein expression was observed by western blot (E), NLRP3, ASC, and caspase-1 protein expression was detected by western blot (F), HIF-1α mRNA was measured by RT–PCR (G), secreted HIF-1α was measured in supernatants by ELISA (H), and HIF-1α protein expression was detected by western blot (I). Data are represented as the mean±SEM of at least three independent experiments. ****P< 0. 0001 or **P<0. 01 compared with the control group; $^{\#\#\#\#}$P<0. 0001 or $^{\#\#\#}$P<0. 001 or $^{\#\#}$P<0. 01 or $^{\#}$P<0. 05 compared with the LPS+MSU group; $^{\&\&\&\&}$P<0. 0001 or $^{\&}$P<0. 01 as shown in the figure.

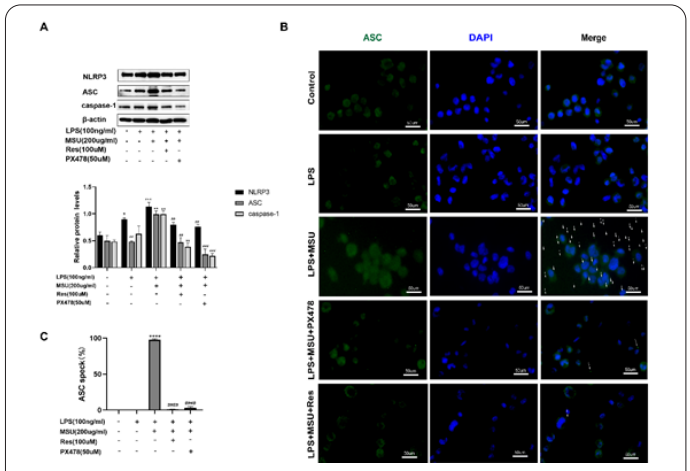

Figure 2. Resveratrol inhibited NLRP3 inflammasome activation by inhibiting HIF-1α. (A) Western blot revealed the presence of NLRP3, ASC, and caspase-1 proteins. (B) Cells were fixed and permeabilized, and immunofluorescent staining was performed for ASC. DAPI (blue fluorescence) was used to stain the cell nucleus, and ASC specks are presented as green fluorescence shown by white arrows. Images were acquired by fluorescence microscopy. (C) ASC specks were statistically analyzed. Data are represented as the mean±SEM of at least three independent experiments. ****P<0. 0001 or ***P<0. 001 or **P<0. 01 or *<0. 05 compared with the control group; $^{\#\#\#\#}$P<0. 0001 or $^{\#\#\#}$P<0. 001 or $^{\#\#}$P<0. 01 or $^{\#}$P<0. 05 compared with the LPS+MSU group.

Resveratrol also reduced the protein expression of HIF-1α and HIF-1α mRNA (Figure 3A, 3E). HIF-1α immunofluorescence staining revealed that resveratrol inhibited HIF-1α expression in a manner similar to that of PX478 (Figure 3B, 3C). In addition, decreased IL-1β protein expression and IL-1β mRNA were observed following the use of PX478, identical to the effect of resveratrol (Figure 3A, 3E). The same results were obtained using ELISA for cell supernatants (Figure 3D).

Resveratrol reduced ankle joint swelling and attenuated inflammatory cell infiltration and synovial hyperplasia in rats with MSU-induced gouty arthritis

Acute gouty arthritis in rats was induced by MSU. The rats were treated with colchicine and various concentrations of resveratrol for 7 days. The model was established on the 5th day to observe the effect of different concentrations of resveratrol on the degree of swelling of the ankle joint and synovial pathology in rats with acute gouty arthritis. As shown in Figures 4A and 4B, ankle swelling in the model group was significantly higher than that in the control group, which indicated that the model was successfully constructed. Meanwhile, the results showed that treatment with different concentrations of resveratrol ameliorated ankle swelling, and higher doses had a considerably greater effect. Moreover, treatment with colchi-

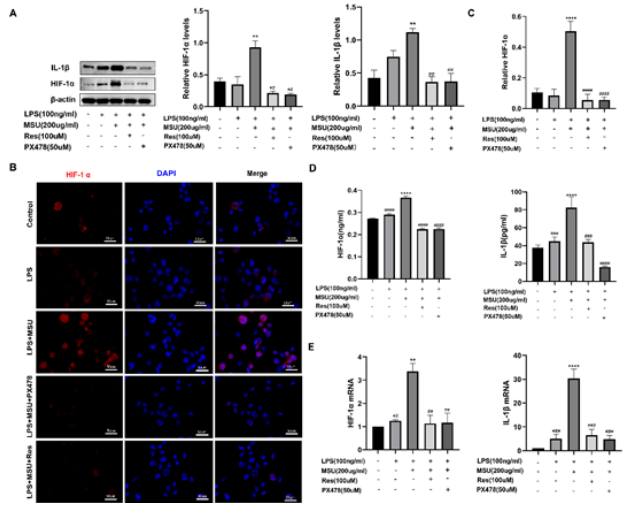

Figure 3. Resveratrol reduces the release of IL-1β by inhibiting HIF-1α. (A) HIF-1α and IL-1β protein expression was detected by western blot. (B and C) Cells were fixed and permeabilized, and immunofluorescent staining was performed for HIF-1a. DAPI (blue fluorescence) was used to stain the cell nucleus, and HIF-1α is presented as red fluorescence. Fluorescence microscopy was used to capture the images (B). The fluorescence intensity data were statistically analyzed (C). (D) Secreted HIF-1α and IL-1β were detected in supernatants by ELISA. (E) HIF-1α and IL-1β mRNA was detected by RT–PCR. Data are represented as the mean ± SEM of at least three independent experiments. ****P<0. 0001 or***P<0. 001 or **P<0. 01 or *<0. 05 compared with the control group; $^{\#\#\#\#}$P<0. 0001 or $^{\#\#\#}$P<0. 001 or $^{\#\#}$P<0. 01 or $^{\#}$P<0. 05 compared with the LPS+MSU group.

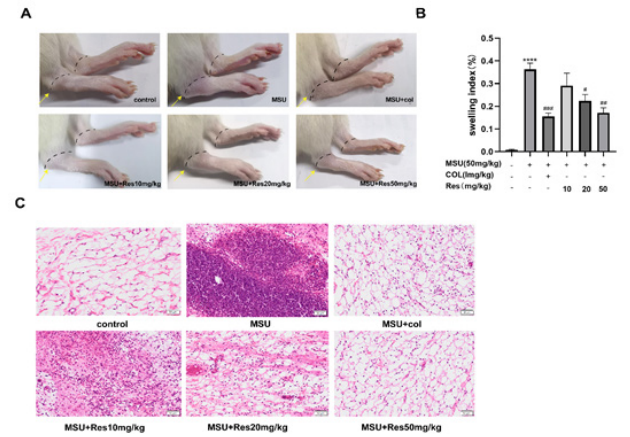

Figure 4. Effect of resveratrol on ankle joint swelling in rats with MSU-induced acute gouty arthritis. (A) Representative images of ankle joint swelling in rats from each group 48 hours after MSU injection. (B) The ankle swelling index was calculated by measuring ankle circumference in rats pre- and post-modeling. (C) Ankle joint tissue samples were excised and stained with HE. The data are presented as the mean ± SEM of at least three separate experiments. ****P<0. 0001 compared with the control group; $^{\#\#\#}$P<0. 001 or $^{\#\#}$P<0. 01 or $^{\#}$<0. 05 compared with the MSU group.

cine led to virtually identical results. We further evaluated inflammatory cell infiltration and histological changes in the ankle joints with HE staining. Figure 4C showed that the infiltration of synovial membranes, synovial hyperplasia, and some neovascularization areas was significantly higher in the model group than in the other groups. The joint tissue sections of the resveratrol treatment groups showed varying degrees of decreased inflammatory cell infiltration, with high-dose resveratrol showing the greatest decrease. Colchicine showed a similar pattern.

Discussion

Acute gouty arthritis is a synovial inflammatory disease characterized by the accumulation of MSU crystals in joints (25). The self-limiting nature of acute flares and the IL-1β pathway are essential in acute gout attacks, suggesting that pro- and anti-inflammatory regulatory pathways are involved in gout (26). In recent years, the prevalence of gout has increased and the affected population has tended to be younger; and recurrent gout attacks have a serious impact on patients' quality of life (27, 28). Colchicine, non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids are currently used to treat acute gout. However, these drugs are associated with varying degrees of adverse effects, including liver damage, nephrotoxicity, and myelodysplasia (11, 29, 30). As a result, there is an immediate need for a drug that is both safe and effective in the treatment of acute gouty arthritis. Many studies have shown the potential efficacy of herbal medicine for gout, reducing adverse effects and improving patient compliance (31, 32), such as Rhizoma Smilacis Glabrae extracts (33), celery seed extracts (34), gallic acid (35). Resveratrol is a known natural polyphenol found in grapes, peanuts, berries, and other plants. It has been studied extensively for its potential to exert immunomodulatory effects by targeting inflammatory cytokines (14). Furthermore, resveratrol has an effect on the recurrent attacks of gouty arthritis (36). Therefore, the current study focused on the potential molecular mechanism of resveratrol's effect on gouty arthritis. Our findings indicated that resveratrol effectively reduced ankle swelling and synovial inflammation in vivo in a rat model of acute gouty arthritis through inhibition of HIF-1α- and NLRP3-derived IL-1β secretion in macrophages.

Macrophages and other innate immune cells play critical roles in the pathogenesis of gouty arthritis (37). The phagocytosis of MSU crystals by macrophages activates the formation of the NLRP3 inflammasome, which results in the release of mature IL-1β, causing localized joint inflammation (6). THP-1 cells were chosen as an in vitro gout model because they have been widely used to study monocyte/macrophage function and the effects of anti-inflammatory drugs in vitro (38). The results showed that both IL-1β mRNA and IL-1β protein levels were increased in MSU-treated THP-1-derived macrophages, indicating an in vitro gout model was successfully constructed. Moreover, resveratrol inhibited the release of IL-1β from MSU-treated THP-1-derived macrophages, consistent with previous findings (17, 39). Resveratrol also considerably enhanced the viability of LPS/MSU-treated THP-1-derived macrophages in vitro, illustrating the protective effect of resveratrol on macrophages. Furthermore, resveratrol effectively alleviated ankle swelling and synovial inflammation in acute gouty arthritis in vivo. When resveratrol was administered at high doses (50 mg/kg/day), the effects were similar to those of colchicine. Colchicine is a first-line clinical drug for acute gout, but it has side effects such as abdominal pain, diarrhea, abnormal liver function, and poor bone marrow hyperplasia, which can lead to renal failure, thus limiting the wide clinical application of colchicine (40). Previous studies have confirmed that colchicine can reduce capillary permeability, inhibit the exudation of inflammatory cells and aggregate and block

the release of macrophages from the cytokines IL-1β, IL-6, and IL-8. Furthermore, in vitro studies have shown that colchicine can reduce NLRP3 inflammasome activation and IL-1β production (15, 29). These findings suggested that resveratrol's therapeutic benefit in gouty arthritis may be strongly associated with the downregulation of inflammatory cytokines, which could be associated with the inhibition of NLRP3 inflammasome activation. Therefore, the mechanism of action of resveratrol was consistent with colchicine, suggesting that it could be used as an alternative drug in the treatment of acute gout.

To better understand the mechanism of resveratrol's anti-inflammatory effect, we investigated its impacts on NLRP3 inflammasome. Phagocytosis of MSU by macrophages can initiate the NLRP3 inflammasome, which is thought to be important in acute gout attacks (15). Here, we demonstrated that resveratrol concentrations above 50 μM could inhibit the MSU-induced protein expression of NLRP3, caspase-1 and ASC in THP-1-derived macrophages. However, one study has shown that resveratrol cannot affect the protein expression of NLRP3 and ASC (17), possibly due to insufficient concentrations of resveratrol. In addition, our immunofluorescence results showed that resveratrol could alleviate ASC oligomerization caused by MSU. We found that resveratrol might reduce the maturation and release of IL-1β by inhibiting the synthesis or assembly of NLRP3 inflammasomes, thereby alleviating MSU-induced gouty arthritis.

Resveratrol can suppress HIF-1α expression by inhibiting HIF-1α protein synthesis or promoting HIF-1α protein degradation via the proteasome (41). Furthermore, it is discovered that HIF-1α mediates the activation of the NLRP3 inflammasome (42), and HIF-1α activation induces the proinflammatory cytokine IL-1β (7). Consequently, we speculated that resveratrol might regulate the inflammatory reaction of gout through HIF-1α expression. Our results suggested that HIF-1α was expressed at a high level in MSU-treated THP-1-derived macrophages. Meanwhile, we found that both resveratrol treatment and the HIF-1α inhibitor, PX478, inhibited the activation of IL-1β. Furthermore, protein expression of NLRP3 inflammasome components was reduced, and ASC oligomerization was inhibited due to resveratrol treatment. These findings indicated that resveratrol inhibited NLRP3 inflammasome activation by suppressing HIF-1α expression, causing a decrease in IL-1β release. Previous studies have indicated that the knockdown of HIF-1α attenuates NLRP3 activation and reduces the release of IL-1β (43), and the conclusions are similar to those in our study. HIF-1α has been shown to play a significant part in the pathogenesis of other joint symptoms, such as rheumatoid synovitis (44), further supporting our perspective.

There are some limitations to this study. First, the rat model we used was initiated by MSU crystals and given that some patients in the clinic have had high uric acid levels for years without a gout attack, clinical trials are required to determine whether the use of resveratrol in patients with gout should be recommended. Second, while studies have shown that resveratrol reduces gouty arthritis by inhibiting the expression of HIF-1α, additional details of the mechanism remain to be discovered.

In conclusion, our study found that resveratrol had a notable therapeutic and preventive effect on an MSU-induced inflammatory model of THP-1 cells and a rat model of gouty arthritis. Its potential mechanism could be to reduce IL-1β maturation and secretion via HIF-1α modulation and inhibition of NLRP3 inflammasome activation. As a result, resveratrol may be an extremely promising candidate for the prevention and treatment of gouty arthritis.

Data Availability

The data in this article can be obtained from the corresponding author under reasonable circumstances.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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