Ferulic acid exhibits anti-inflammatory effects by inducing autophagy and blocking NLRP3 inflammasome activation

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Abstract
Background Inflammation is involved in the healing process; however, when inflammation is overactivated, multiple diseases can occur. The continued discovery of new anti-inflammatory drugs is crucial in the treatment of inflammation-linked diseases.
Objectives Ferulic acid (FA), a precursor necessary for lignan synthesis, is widely distributed in plant-based whole foods and is a strong antioxidant. However, the effect of FA on the expression level of inflammatory factors in macrophages has not been fully clarified. The current study aimed to explore the anti-inflammatory effect and mechanism of ferulic acid.
Results The results showed that THP-1 cells were induced to differentiate into macrophages by Phorbol-12-myristate-13-acetate (PMA), and THP-1-derived macrophages were stimulated by LPS to establish an inflammatory cell model. Compared with the control group, low (5 μmol·mL⁻¹), medium (10 μmol·mL⁻¹), and high (20 μmol·mL⁻¹) concentration ferulic acid groups have decreased cell viability and increased apoptosis rate in a dose-dependent manner. FA reduced the transcriptional levels of Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). Importantly, FA-induced autophagy and inhibited NLRP3 inflammasome activation. 3-MA (a widely used autophagy inhibitor) enhanced the secretion of TNF-α, IL-6 and IL-1β. Moreover, autophagy inhibition by 3-MA resulted in increased proteins expression associated with NLRP3 inflammasome signaling pathway. Besides, the inhibition of inflammasome activation by MCC950 reduced the expression of TNF-α, IL-6 and IL-1β.
Conclusion It is concluded that FA enhanced autophagy, inhibited the activation of NLRP3 inflammasome and reduced the expression and release of inflammatory factors.

Keywords Ferulic acid · Macrophage · Autophagy · NLRP3 inflammasome · Inflammatory factor

Introduction

Inflammation is vital to defend against tissue injury, infection or toxic agents and involves recruitment of phagocytes (macrophages and neutrophils) and the secretion of various cytokines (Medzhitov 2008). During an inflammatory process, macrophages are one of the most important phagocytes, which can participate in the development of a variety of diseases by secreting a large number of cytokines, such as TNF-α, IL-6 and IL-1β (Medzhitolv 2010). Such inflammatory mediators can act on both immune cells and non-immune cells to protect the body from harmful substances and pathogens by inducing an immune response (Medzhitolv 2008). Persistent inflammation has been associated with several diseases, such as cancers, arteriosclerosis, rheumatoid arthritis, asthma and Alzheimer’s disease (Back et al. 2019; DiDonato et al. 2012; Glass et al. 2010).

THP-1 is a human monocytic cell line that commonly used in the laboratory and can differentiate into macrophages by inducing with phorbol 12-myristate 13 acetate (PMA) (Lund et al. 2016; Biriken et al. 2018). Macrophages express various pathogen recognition receptors, including Toll-like receptors (TLRs) (Gruber and Leifer 2020). Lipopolysaccharide (LPS) can be recognized by TLR-4 and further activates...
the specific signaling pathways downstream of TLR-4, leading to the activation of NF-κB and proinflammatory genes expression (Liu et al. 2017).

Inflammasomes are widely distributed cytosolic sensors which can sense metabolic imbalances or pathogen infections and trigger the release of proinflammatory cytokines (Guo et al. 2015). Currently, one of the most well-studied inflammasomes is NOD-like receptor-family pyrin domain-containing 3 (NLRP3) inflammasome (Broz and Dixit 2016). According to research findings, the full activation of NLRP3 inflammasomes requires two signals (Franchi et al. 2012). The first signal involves the interaction between TLRs and their ligands, the activation of NF-κB and the synthesis of pro-IL-1β and NLRP3 (Franchi et al. 2012). The second stimulus leads to caspase-1 autoactivation, the cleavage of pro-IL-1β, and the release of mature IL-1β (Franchi et al. 2012). Appropriate concentration of lipopolysaccharide has been confirmed to provide two activation signals in human monocytes and macrophages and led to IL-1β secretion (Petes et al. 2017; Tang et al. 2019; Zhao et al. 2019). As a multifunctional inflammatory factor, IL-1β performs both immunity and anti-infective functions by activating the expression of other immune genes and recruiting lymphocytes to the site of infection (Dinarello 2011).

Autophagy plays a critical role in maintaining cell homeostasis by means of circulating materials and energy inside the cells in an environment that is not conducive to cell growth (Glick et al. 2010). The NLRP3 inflammasome is an important part of the innate immune system (Guo et al. 2015). Numerous studies have shown that there is a complex regulatory relationship between autophagy and inflammasome (Cao et al. 2019). Autophagy has been found to inhibit NLRP3 inflammasome activation in some cases and activate NLRP3 inflammasome activation in other cases (Cao et al. 2019). Meanwhile, NLRP3 inflammasome has been reported to affect autophagy (Cao et al. 2019). In this study, the complex regulatory relationship between autophagy and NLRP3 inflammasome was further clarified.

Ferulic acid (FA) is a polyphenolic compound that can be found in a variety of plants, for example grains, vegetables and fruits (de Oliveira et al. 2015). FA has been confirmed to have various biological activities, such as antimicrobial, anticarcinogenic, antioxidant, hepatoprotective, and vasodilatory actions (Amic et al. 2020; Zhang et al. 2019; Zhou et al. 2020). FA has been reported to have anti-inflammatory effects in several studies. Ferulic acid has been reported to inhibit bovine endometrial epithelial cells against LPS-induced inflammation via suppressing NK-kappa B and MAPK pathway (Wu et al. 2021). Ferulic acid also ameliorates hepatic inflammation and fibrotic liver injury by inhibiting PTP1B activity and subsequent promoting AMPK phosphorylation (Wu et al. 2021). Meanwhile, FA has been confirmed to induce autophagy and alleviates lipotoxicity-induced hepatocellular death through the SIRT1-regulated autophagy pathway (Xu et al. 2021). Ferulic acid protects cardiomyocytes from TNF-alpha/cycloheximide-induced apoptosis by regulating autophagy (Li et al. 2020). FA mitigated oxygen–glucose deprivation (OGD)-induced mitochondrial oxidative damage by punctate-mitochondria-dependent autophagy (Chen et al. 2017). But the relationship between FA-mediated anti-inflammatory effects and autophagy induction has not been clearly elucidated.

In this study, we used a cell inflammatory model to investigate the anti-inflammatory activity of ferulic acid and its related mechanism.

**Materials and methods**

**Reagents**

RPMI-1640 culture medium and foetal bovine serum were obtained from Gibco. Ferulic acid (FA), lipopolysaccharide (LPS), Phorbol-12-myristate-13-acetate (PMA), thiazoyl blue tetrazolium bromide (MTT), and 3-methyladenine (3-MA) were purchased from Sigma-Aldrich. MCC950 was purchased from Abmole Bioscience Inc. DCFH-DA was purchased from Beyotime Biotechnology Co., Ltd. The reverse transcription kits and SYBR Premix Ex Taq were purchased from Takara Biomedical Technology. The antibodies against Microtubule Associated Protein 1 Light Chain 3 Beta (LC3), Sequestosome 1 (p62), Beclin-1, NLRP3, pro-caspase-1, caspase-1, pro-Interleukin 1 Beta (IL-1β), IL-1β and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) were purchased from ABclonal Technology. The Human IL-6 ELISA Kit, Human IL-1β ELISA kit and human TNF-α ELISA Kit were purchased from Multisciences (Lianke) Biotech, Co., Ltd. All other reagents used were obtained from local commercial sources.

**THP-1 cell differentiation**

The THP-1 (human monocyte-macrophage cell line; ATCC) was cultured in RPMI-1640 medium supplemented with 10% foetal calf serum, HEPES (10 mM) and β-mercaptoethanol (0.05 mM) at 37 °C in a CO₂ incubator. The THP-1 cells were distributed into cell cultured plate (at a concentration of 2 × 10⁵ cells/mL) and cultured with 25 nM PMA for 48 h (Lund et al. 2016). After discarding the medium containing PMA, the THP-1-derived macrophages (Mφs) were first...
The THP-1-derived macrophages were cultured (1 × 10^6 cells/well) and remained untreated for 24 h and then cultured with 100 ng/mL LPS for 4 h (37 °C and 5% CO2). Then, the cell viability, expression of IL-6, IL-1β, and TNF-α and ROS production were determined.

### MTT assay

The THP-1-derived macrophages were induced differentiation and maintained as described above; then, the culture medium was removed, and 100 μL of MTT solution (2 mg/mL) were added per well for 4 h to allow MTT uptake. Subsequently, the excess MTT was discarded, and 100 μL of dimethyl sulfoxide were added to dissolve the formed formazan. The absorbance was measured on a Thermo Scientific Microplate Reader with wavelengths of 490 nm.

### Enzyme-linked immunosorbent assay (ELISA)

After treating macrophages with ferulic acid and LPS as described above, the culture supernatants were collected and the amounts of TNF-α, IL-6 and IL-1β in the supernatant were measured using human ELISA kits in accordance with the manufacturer’s use instructions.

### Real-time quantitative RT-PCR (RT-qPCR)

The THP-1-derived macrophages were cultured (1 x 10^6 cells/well) or remained untreated for 24 h and then cultured with 100 ng/mL LPS for 4 h (37 °C and 5% CO2). Then, the cell viability, expression of IL-6, IL-1β, and TNF-α and ROS production were determined.

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\Delta \Delta CT = (CT \text{ target gene - } CT \text{ Gapdh gene}) \text{ treatment group } - \text{(CT target gene - CT Gapdh gene) control group. At least three biological samples were tested for per gene or treatment and a melt curve analysis was used to evaluate the specificity of the primers.}
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### Cell apoptosis and ROS content were determined by flow cytometry

Cells in each treatment group were collected and washed with PBS. Cells were resuspended with 500 μL 1× binding buffer, added Annexin V-FITC (5 μL)/PI (10 μL) and incubated in dark for 10 min before analysing by flow cytometry.

DCFH-DA is a cellular permeable probe for the detection of intracellular reactive oxygen species (ROS). DCFH-DA was diluted to a suitable concentration (10 μM) with serum-free medium. Then, the medium in the culture plate was discarded and an appropriate volume of DCFH-DA was added to cover the cells. Usually, no less than 1 mL of DCFH-DA was added to one well (six-well plate). The cells were incubated in an incubator at 37 °C for 20 min. The cells were washed twice with serum-free medium three times and LPS (100 ng/mL) was added to the cell culture supernatant with or without the ferulic acid pretreatment. After 1 h, the cells were collected and the fluorescence intensity was detected by flow cytometry.

### Western blot

The treated macrophages were washed once with ice-cold PBS and lysed with RIPA lysis buffer supplemented with protease inhibitors. Then, the cell lysates were collected and centrifuged for 10 min at 13,000 rpm at 4 °C to clear cellular debris deposits, and the protein concentrations were measured with BCA protein assay. The samples were boiled at 100 °C for 10 min in loading buffer before the electrophoretic separation. The cell lysates were separated using 8–12% SDS-PAGE and then transferred onto PVDF membranes. After blocking the membranes with 5% nonfat milk in TBST at room temperature for 2 h, the membranes were incubated with LC3, p62, Beclin-1, NLRP3, pro-caspase-1, caspase-1, pro-IL-1β, IL-1β and GAPDH antibodies and corresponding secondary antibodies. Finally, the protein–antibody complex signals were detected by enhanced chemiluminescence using a commercial kit according to the manufacturer’s suggested protocols. ImageJ software was used to quantitate the intensity of the bands.

### Data analysis

All results are presented as the means ± standard deviation. In the statistical analysis, one-way analysis of variance (one-way ANOVA) was performed using GraphPad Prism 6.0 software. A significant difference was defined as \( P < 0.05 \).
Results

The effect of ferulic acid on the activity of THP-1-derived macrophages

To determine whether monocytes differentiate into macrophages, we continuously observed the changes in cell morphology through microscopy. As shown in Fig. 1A, as the PMA induction time increased, the THP-1 cells changed from an elliptical suspension state to an irregular attachment state with a large number of pseudopods, indicating that the THP-1 cells had been successfully induced into macrophages (Lund et al. 2016). To further clarify the cytotoxic effect of ferulic acid, the THP-1-derived macrophages were exposed to different concentrations of FA (5, 10, 20 µM) for 24 h. Compared with the control group, low, medium, and high concentration ferulic acid groups have decreased cell viability and increased apoptosis rate in a dose-dependent manner (\(P < 0.05\)) (Fig. 1, Table 1).

FA suppresses LPS-induced TNF-α, IL-6 and IL-1β expression and ROS production in vitro

TNF-α, IL-6 and IL-1β are major inflammatory mediators contributing to the pathogenesis of inflammatory diseases. THP-1-derived macrophages constitutively expressed lots of inflammatory cytokines, including TNF-α, IL-6 and IL-1β, at low levels. To elucidate whether FA could regulate the expression of inflammatory mediators, the total mRNA extracted from the treated macrophages was subjected to a qPCR analysis. The experimental groups were pretreated with 20 µM FA and then stimulated with LPS at 100 ng/mL for 4 h. Non-stimulated cells were used as a negative control and LPS-stimulated cells were used as a positive control. As shown in Fig. 2, the addition of LPS led to a remarkably increase in the mRNA expression of TNF-α, IL-6 and IL-1β, and the pretreatment with FA obviously decreased the mRNA expression of TNF-α, IL-6 and IL-1β. We also detected the impact of FA on ROS formation and found that FA reduced ROS formation in macrophages activated by LPS in a dose-dependent manner (Table 2).

| Group  | Concentration (µmol mL\(^{-1}\)) | Cell viability (%) | Apoptosis rate (%) |
|--------|---------------------------------|--------------------|-------------------|
| Blank control | 0 | 98.63 ± 5.07 | 2.63 ± 0.28 |
| FA-L | 5 | 96.96 ± 1.77 | 2.93 ± 0.12 |
| FA-M | 10 | 90.20 ± 2.24 | 6.10 ± 0.96* |
| FA-H | 20 | 82.41 ± 3.30* | 11.50 ± 1.02** |

Low concentration; M medium concentration; H high concentrations. Compared with blank control group, *\(P < 0.05\), **\(P < 0.01\)

FA-induced autophagy regulates inflammatory cytokine release

Autophagy is a process that maintains cell homeostasis by degrading certain cell contents and has been confirmed to play a key role in inflammation, programmed cell death, and

![Fig. 1](image-url)  
**Fig. 1** A Morphology of THP-1-derived macrophages treated with PMA for 12 and 24 h as observed by microscopy (200×). B Flow cytometry was used to detect the apoptosis rate of THP-1-derived macrophages in each group. Values are means ± SD of three independent experiments.
adaptive immunity (Glick et al. 2010). To further clarify the possible relationship between autophagy and inflammation, we detected the expression levels of autophagy-related proteins in treated cells. As shown in Figs. 3B and 4A, no significant difference could be observed in the expression ratio of LC3, p62 and Beclin-1 between the LPS exposure group and the control group. However, the protein expression levels of LC3-II and Beclin-1 were significantly increased in the FA-pretreated cells, indicating the occurrence of autophagy.

To further elucidate the effect of autophagy on inflammation, 3-MA (an autophagy inhibitor) was applied. As demonstrated in Figs. 3 and 4A, 3-MA obviously decreased LC3-II and Beclin-1 expression. Moreover, 3-MA reversed the reduction in inflammatory factor (TNF-α, IL-6 and IL-1β) release induced by FA, suggesting that autophagy plays a vital role in regulating inflammation (Fig. 4C).

FA inhibits LPS-induced activation of the NLRP3 inflammasome

NLRP3 inflammasome, an intracellular signaling platform that induces the activation of inflammatory caspases and the release of interleukin-1β, is an important part of innate immunity (Zhao and Zhao 2020). To further clarify the effect of FA on inflammasome activation, we examined the protein expression of the inflammasome signalling pathway. As expected, the protein expression levels of NLRP3, activated caspase-1 and IL-1β were significantly increased after the LPS induction. However, the high level of IL-1β induced by LPS (Fig. 5A, $P < 0.01$) was dramatically decreased in the 20 µM FA pretreatment group. Moreover, the increased expression of NLRP3 and caspase-1 triggered by LPS was also attenuated in the FA pretreatment group. These results strongly confirmed that FA inhibited the LPS-triggered activation of the NLRP3 inflammasome in the THP-1-derived macrophages.

FA-mediated inhibition of NLRP3 inflammasome activation is mediated by autophagy induction

To further explore the role of inflammasome in the production of inflammatory factors, we used the NLRP3 inflammasome inhibitor MCC950. As illustrated in Fig. 6, the increased levels of NLRP3, activated caspase-1 and IL-1β induced by LPS were attenuated dramatically in the MCC950 pretreatment group (Fig. 6A, B, $P < 0.01$). Moreover, a marked decrease in the release of TNF-α, IL-6 and IL-1β expression was detected (Fig. 6C–E, $P < 0.01$).

This research found that FA reduced the expression of inflammatory factors by inducing autophagy. We also found that FA inhibits LPS-induced activation of the NLRP3 inflammasome. To further clarify the relationship between autophagy and the NLRP3 inflammasome in LPS-treated THP-1-derived macrophages, we used the autophagy inhibitor 3-MA and detected the protein expression of NLRP3 inflammasome-related proteins. As illustrated in Fig. 6A, B, the protein expression of NLRP3, activated caspase-1 and IL-1β was markedly decreased in the FA-treated cells, and 3-MA markedly increased NLRP3, activated caspase-1 and

Table 2 Effect of ferulic acid on the expression of ROS in THP-1-derived macrophages (\(\bar{X} \pm s\))

| Group       | Concentration (\(\mu\text{mol mL}^{-1}\)) | Relative DCF fluorescence (%) |
|-------------|------------------------------------------|-----------------------------|
| Blank control | –                                       | 98.00 ± 1.16                |
| Model       | –                                       | 125.0 ± 2.89***             |
| FA-L        | 5                                       | 119.7 ± 2.60                |
| FA-M        | 10                                      | 102.3 ± 1.45##              |
| FA-H        | 20                                      | 102.3 ± 3.71##              |

Compared with blank control group, ***$P < 0.001$; Compared with model group, ##$P < 0.01$
IL-1β protein expression (Fig. 6A, B, \( P < 0.01 \)). To sum up, the data show that FA may downregulate the expression of inflammatory factors by inducing autophagy and inhibiting the activation of the NLRP3 inflammasome.

**Discussion**

Inflammation is an important defense response of the innate immune system against various injurious factors (Medzhitov 2010). During inflammation, a series of local reactions centered on the vascular system limited and eliminated injury factors, promoted the healing of damaged tissue and involves a network of cellular responses and cytokines, including IL-1β, IL-6, and TNF-α (Medzhitov 2008). Generally speaking, the release of these inflammatory mediators is beneficial to the body, but excessive release of inflammatory mediators is harmful to the body, and is even closely related to a variety of inflammation-related diseases (Back et al. 2019; Mahmudpour et al. 2020). IL-6 is an important inflammatory mediator with a pleiotropic effect on cancer progression, inflammation and immune response (Tanaka et al. 2014). IL-6 is mainly synthesized and secreted by immune cells (T cells, monocytes macrophages) and non-immune cells (fibroblasts, endothelial cells) during infection and tissue injury (Mihara et al. 2012). Interleukin-1β is a vital mediator of inflammatory response (Gabay et al. 2010; Lopez-Castejon and Brough 2011). Tumor necrosis factor-α (TNF-α) is an inflammatory mediator produced by mononuclear macrophages during inflammation (Atzeni et al. 2020; Karki et al. 2021). This cytokine is not only involved in multiple intracellular signaling events leading to necrosis or apoptosis, but also important in the fight against infection and cancer (Atzeni et al. 2020; Karki et al. 2021). In this study, the addition of LPS led to a remarkably increase in the mRNA expression of TNF-α, IL-6 and IL-1β, and the pretreatment with FA obviously decreased the mRNA expression of TNF-α, IL-6 and IL-1β.

Ferulic acid is a kind of widely distributed phenolic acid, which is often cross-linked with lignin and polysaccharide to form part of the cell wall of plants (de Oliveira et al. 2015). It was found that ferulic acid had strong antioxidant activity, and could scavenging hydrogen peroxide, hydroxyl radical and superoxide radical (Zhao and Moghadasian 2008). At the same time, ferulic acid has also been proved to have antibacterial and anti-inflammatory effects (Zhao and Moghadasian 2008). FA also has been confirmed to be a promising substance protecting against high-fat diet-induced weight
Fig. 4 FA inhibited inflammation by enhancing autophagy. A, B THP-1-derived macrophages were pretreated with FA (20 µM) or 3-MA (1 mM) before stimulation with LPS (100 ng/mL) for another 4 h, and LC3, p62, and Beclin-1 protein expression was detected by a Western blot analysis. C–E The cytokines TNF-α, IL-6, and IL-1β were determined using ELISA kits. Values are the means ± SD of three independent experiments. ^^P < 0.01, ^^^P < 0.001, compared with the control group; *P < 0.05, **P < 0.01, compared with the LPS-treated group; #P < 0.05, ##P < 0.01, compared with the LPS + FA-treated group

Fig. 5 LPS-induced activation of the NLRP3 inflammasome in THP-1-derived macrophages is attenuated by FA treatment. A THP-1-derived macrophages were treated with LPS (100 ng/mL) for 4 h in the presence or absence of pretreatment with FA (20 µM) for 24 h. The protein levels of NLRP3, pro-caspase-1, caspase-1, pro-IL-1β and IL-1β were evaluated by a Western blot analysis. GAPDH was used as a loading control. B Densitometry analysis of the effects of FA on the expression of NLRP3, caspase-1 and IL-1β. Values are the means ± SD of three independent experiments. *P < 0.05, **P < 0.001, compared with the control group; †P < 0.05, ‡P < 0.01, §P < 0.001, compared with the LPS-treated group; ns no significant difference
Our studies demonstrated that FA disturbed the pro-inflammatory activity of macrophages by decreasing TNF-α, IL-6 and IL-1β secretion induced by LPS.

The inflammasomes are a class of macromolecular complexes that exist in the cytoplasm and are capable of sensing pathogenic microorganism infection, tissue injury and metabolic imbalance (Broz and Dixit 2016; Guo et al. 2015). NLRP3 inflammasome is the most widely studied inflammasome, which is mainly composed of NLRP3, ASC (apoptosis-associated speck-like protein containing a CARD) and procaspase-1 (Broz and Dixit 2016; Zhao and Zhao 2020). When receptors perceive signals from pathogen infection, tissue damage, or metabolic imbalance, they recruit ASC and pro-caspase-1 to form inflammasomes, which in turn activate caspase-1 and induced the secretion of IL-1β and IL-18 (Zhao and Zhao 2020). At present, it is believed that the activation of inflammasome may be related to the occurrence of a variety of diseases, and further study of inflammasome may provide a new theoretical basis for the prevention and treatment of related diseases (Guo et al. 2015). In this study, it was found that FA down-regulated the protein expression...
of NLRP3, activated caspase-1 and IL-1β and inhibited the LPS-triggered activation of the NLRP3 inflammasome. Meanwhile, when MCC950 was used to inhibit the activation of NLRP3 inflammasomes, the release of TNF-α, IL-6 and IL-1β was significantly reduced. These results suggest that FA may downregulate the expression of TNF-α, IL-6 and IL-1β by inhibiting the activation of NLRP3 inflammasomes.

Reports have indicated that NLRP3 inflammasome activation needs a second signal delivered from ROS production, potassium efflux or calcium influx (Franchi et al. 2012). To explore whether FA modulates the second signal required for NLRP3 activation, FA-pretreated THP-1-derived macrophages were stimulated with LPS, and then, ROS production was detected. The results showed that the FA pretreatment reduced LPS-induced intracellular ROS production (Fig. 2D). These results suggest that FA downregulates the activation of NLRP3 inflammasomes by inhibiting the expression of ROS.

Autophagy is a biochemical process that takes place in cells all the time (Glick et al. 2010). Its function is to encapsulate damaged organelles and misfolded proteins through the formation of autophagosomes and transport them to lysosomes for degradation (Glick et al. 2010; Parzych and Klionsky 2014). Many molecules have been shown to be involved in autophagy, including LC3, Beclin-1 and p62 (Glick et al. 2010). In this study, the effect of FA on macrophage autophagy was explored, and FA-induced autophagy by increasing LC3 and Beclin-1 expression and decreasing p62 expression (Fig. 4). To further clarify the relationship between autophagy and inflammation, 3-MA was selected to block autophagy. In this study, to further determine the appropriate concentration of 3-MA, the THP-1-derived macrophages were exposed to different concentrations of 3-MA (0, 200 µM, 400 µM, 800 µM, 1 mM, 2 mM, 4 mM, 8 mM) for 4, 12 and 24 h. Compared with the control group, 2, 4 and 8 mM 3-MA groups have significantly decreased cell viability ($P < 0.05$) (Fig. 3A). Therefore, in subsequent studies, concentrations of 0, 200 µM, 400 µM, 800 µM and 1 mM were selected. To further clarify the effect of 3-MA on autophagic protein expression, we detected the expression levels of autophagy-related proteins in treated cells. As shown in Fig. 3B, with the increase of 3-MA concentration (0, 200 µM, 400 µM, 800 µM, 1 mM), LC3-II and Beclin-1 protein expression level decreased significantly. Considering the cytotoxic effect and inhibition efficiency of autophagy, 800 µM or 1 mM may be a reasonable concentration of 3-MA. More importantly, the autophagy inhibitor 3-MA activated the NLRP3 inflammasome pathway and enhanced TNF-α, IL-6 and IL-1β release (Fig. 6). It is suggested that FA, on one hand, inhibits the expression of ROS and on the other hand, induces autophagy to inhibit the activation of inflammasome, and ultimately downregulates the expression and release of TNF-α, IL-6 and IL-1β.

In conclusion, our study suggested that FA enhanced autophagy, inhibited the activation of NLRP3 inflammasome and reduced the expression and release of inflammatory factors. Our study provides evidence that FA might be a potential therapeutic agent for inflammatory diseases by virtue of its anti-inflammatory activity (Fig. 7).
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Author contributions YL, LS, WQ were responsible for designing research studies, conducting experiments and acquiring data. YL and YS were responsible for providing reagents, designing research studies, analyzing data, and writing the manuscript. All authors have read and approved the manuscript.

Declarations

Conflict of interest Yongjuan Liu declares she has no conflict of interest. Lu Shi declares she has no conflict of interest. Wenqiong Qiu declares he has no conflict of interest. Yingying Shi declares she has no conflict of interest.

Ethical statement The study includes only laboratory studies on a cell line. No ethical or institutional approval was required.

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