Imperatorin alleviated NLRP3 inflammasome cascade-induced synovial fibrosis and synovitis in rats with knee osteoarthritis

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Research article

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Abstract

Background: To clarify the therapeutic potential of imperatorin (IMP) in knee osteoarthritis (KOA).

Methods: Thirty 3-month-old SD male rats were randomly divided into Normal group, monosodium iodoacetate (MIA) group and MIA+IMP group. Their synovial tissues were subjected to histopathological analysis. Primary synovial fibroblasts obtained from additional normal rats were treated by lipopolysaccharide (LPS) and then IMP. The mRNA and protein expressions of factors related to synovitis and synovial fibrosis were detected by qRT-PCR and Western blotting, respectively. The levels of inflammatory factors IL-1β and IL-18 were measured by ELISA.

Results: IMP reduced HIF-1α, NLRP3 inflammasome expression and IL-1β, IL-18 production in synovial fibroblasts induced by LPS. IMP also down-regulated synovial fibrosis markers. *In vitro* study revealed that MIA-induced synovitis and synovial fibrosis were relieved by IMP.

Conclusion: IMP exerts anti-inflammatory effects associated with synovitis and synovial fibrosis. It reduces the production of pro-inflammatory mediators and cytokines and inhibits TGF-β1, TIMP-1 and VEGF expressions that promote synovial fibrosis.

Background

Knee osteoarthritis (KOA) is a common and disabling condition that represents a substantial and increasing health burden with notable implications for the individuals affected, health-care systems, and wider socioeconomic costs [1,2]. Synovial fibrosis is important pathological processes characterized by abnormal deposition of extracellular matrix (ECM), as well as cell migration and proliferation in the occurrence and development of knee osteoarthritis. Synovial fibrosis is very common in KOA, which are the main causes of pain and joint stiffness. Up-regulation of fibrogenic factors, such as TGF-β1 [3], are signs of the development of synovial fibrosis. TGF-β1 is the most widely known fibrosis factor, and plays a key role in many profibrotic processes, including promoting tissue matrix metalloproteinase (TIMP) expressions [4]. TIMPs are found to be elevated in some diseases related to fibrosis, for example liver fibrosis, as well as in the synovium of mice with OA and human end-stage OA patients. In addition, vascular endothelial growth factor (VEGF) is an effective stimulator of angiogenesis and may also promote synovial fibrosis through extravasation [5]. These three are confirmed to be fibrogenic factors [6].

KOA is a complex disease, the pathogenesis of it involving not only mechanical but inflammatory and metabolic factors, leading to destruction and failure of the knee joints [7]. Each common osteoarthritis risk factor can lead to different mechanisms of knee osteoarthritis, such as increased inflammatory components [8] and oxidative stress. In addition, the extended metabolic activity characteristics of knee synovium contribute to imbalance of oxygen homeostasis and enhance hypoxia in the microenvironment [9]. Hypoxia-induced factor (HIF)-1α is recognized as a major regulator of hypoxia signaling, which mediates the adaptive response of cells to hypoxia by activating the transcription of genes encoding proteins. HIF-1α expression can be triggered in an inflammatory microenvironment even under normoxic

conditions [10]. Clinical studies have shown that HIF-1α levels in serum, synovial fluid, and articular cartilage in patients with KOA are associated with progressive joint damage. It can be used as a biomarker for KOA progression and prognosis. The previous studies have already shown that HIF-1α is associated with upregulation of genes encoding pro-inflammatory cytokines and growth factors, thereby activating fibroblasts and mediating fibrosis. Besides, the NLRP3 inflammasome can be activated by HIF-1α [11]. The NLRP3 inflammasome has been implicated in the pathogenesis of a number of arthritic disorders, producing proinflammatory cytokines and degradative enzymes, such as interleukin-1 beta (IL-1β) which drive cartilage degeneration and synovial inflammation [12]. Inhibiting NLRP3 inflammasome can alleviate many types of fibrosis, especially synovial fibrosis [13,14].

Imperatorin (IMP) is a secondary metabolite of plants. It is one of furanocoumarin derivatives and is widely used in many traditional Chinese herbal medicines (e.g. *Angelica dahurica*) which are used for treatment of KOA. It has the effects of antitumor [15], antibacterial [16], anti-inflammatory [17], but little is known about its action in the suppression of synovial inflammatory and fibrosis. Also, IMP is one of the active ingredients in “Yiceng”, a layer used to treat KOA [18]. Therefore, in this study, we examined the effects of IMP on synovial fibrosis provoked by monosodium iodoacetate (MIA) *in vivo*, and inflammatory model in primary synovial fibroblasts induced by lipopolysaccharide (LPS) *in vitro*. The therapeutic effect of IMP on synovitis and synovial fibrosis reveals it as a potential candidate for drug development.

Materials And Methods

Reagents and antibodies

IMP (purity>99%) was purchased from Yuanye (Shanghai, China). MIA and dimethyl sulfoxide were obtained from Sigma (St Louis, USA). Antibodies against NLRP3, ASC, Caspase-1, VEGF, TIMP1, TGF-β1 and HIF-1α were purchased from Abcam (Cambridge, UK). HRP-conjugated affinipure goat anti-rabbit IgG(H+L) (Proteintech Group, Inc., SA00001-2, 1:20000), HRP-conjugated affinipure goat anti-mouse IgG(H+L) (Proteintech Group, Inc., SA00001-1, 1:20000). ECL luminescent liquid (Shanghai Tianneng, 180-5001), protein marker (Shanghai Tianneng, 180-6003), BCA protein assay kit (Thermo Fisher, 23227). bovine serum albumin, Fetal bovine serum (FBS), Dulbecco’s modified eagle’s medium (DMEM), TRIzol and 0.25% trypsin-EDTA were purchased from Gibco (Life Technologies Corp., California, USA). TransStart Green qPCR SuperMix was obtained from Takara (Dalian, China). The primers and rat GAPDH Endogenous Reference were supplied by Sangon Biotech (Shanghai, China). Enzyme linked immunosorbent assays (ELISA) kits for IL-1β and IL-18 were supplied by Invitrogen (Life Technologies Corp., California, USA). All other chemicals were of reagent grade.

Animals

Thirty 3-month-old SD male rats, weight ranging from 250-290 g, 10 for each group (provided by Beijing Vital River Laboratory Animal Technology Co., Ltd.), were used for experimental KOA studies. Animals were maintained in a specific pathogen-free laminar-flow housing apparatus under controlled temperature, humidity, and 12 h light/dark regimen. All animal protocols were approved by the Animal

Care and Use Committee of the Nanjing University of Chinese Medicine. All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Induction of KOA and Drug Administration

The rat osteoarthritis model was made according to a previous literature [6]. Thirty rats were randomly divided into three groups (Normal group, MIA group, MIA+IMP group). For Normal group, injection of 0.9% saline into articular joint was performed; for MIA group, 2 mg MIA dissolved in 50 μl 0.9% saline; for MIA +IMP group, we chose 5 mg/kg/day as oral administration concentration from 2 weeks after injection as previously described [19]. IMP was dissolved in 0.5% carboxymethylcellulose sodium (CMC-Na), and 0.5% CMC-Na was given by intragastric administration alone in sham group and MIA group every day for 6 weeks until the rats were sacrificed. All rats were sacrificed after eight weeks' post-injection and the synovial tissues were processed for histological analysis and further experiments.

Histopathological analysis

For hematoxylin and eosin (H&E) staining, synovial tissues were frozen and fixed in 4% paraformaldehyde, soaked in 0.5 M EDTA, embedded in paraffin for routine H&E staining and the paraffin blocks were sectioned at a thickness of 5 μm.

Isolation and primary culture of synovial fibroblasts

Primary rat synovial fibroblasts were obtained from additional normal rats. In brief, synovial tissues were washed 2-3 times with phosphate-buffered saline and then minced into pieces of 2-3 mm^2 and digested in 0.1% collagenase type II (Sigma Aldrich, St. Louis, MO, USA) for 30 min. Following cell dissociation, the samples were filtered through a cell strainer. After dissociation, fibroblasts were pelleted by centrifugation at 1500 rpm for 4 min and cultured in DMEM supplemented with 10% FBS (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and antibiotics (100 U/ml penicillin, 100μg/ml streptomycin). Cells were cultured at 37°C in a humidified 95% air and 5% CO_2 atmosphere. Passages 3-6 of the synovial fibroblasts were used for the experiments.

Fibroblasts were stimulated with LPS (5 μg/ml) in DMEM for 6 h to stimulate the inflammatory response and activate the NLRP3 inflammasome. The fibroblasts exposed to DMEM with same volume of saline served as control. Before administration of LPS, IMP were used for 24 h or 48 h for continued experiments.

Synovial extraction and preservation in rats

After 14 days of modeling or 14 days of treatment, the rats were sacrificed by CO_2 asphyxia method, and the rat knee joint hair was removed. The ligament was incised on both sides of the patellofemoral ligament. The upper edge of the humerus was transversely cut to the distal end of the quadriceps muscle until the femur. The ophthalmologist picked up the free tibia and its surrounding tissue and opened it to

the distal end. The pale yellow translucent synovial membrane was seen. The synovial tissue was carefully cut with a surgical blade. Paraformaldehyde was preserved for pathological sectioning, and the rest was placed in a cryotube at -70°C.

Real-time PCR

RNA was isolated from synovial tissues and fibroblasts with Trizol (Invitrogen, CA, USA), respectively. The reverse transcription was performed by using a first strand cDNA synthesis kit (Takara, Otsu, Japan) according to manufacturer's instructions. qPCR was performed using Premix Ex Taq SYBR-Green PCR (Takara) according to manufacturer's instructions on an ABI PRISM 7300 (Applied Biosystems, Foster City, CA, USA).

Primer was designed and synthesized by Shanghai Biotechnology Service Co. Ltd. in accordance with the gene sequence in GenBank gene sequence design, together with Oligo v6.6. Sequences for primers shown in Table 1. The mRNA level of individual genes was normalized to GAPDH and calculated by the $2^{-\Delta\Delta Ct}$ method.

Western blotting

Dissect the synovial tissue, weigh and mix with RIPA lysate. The samples were centrifuged at 15,000 r/min for 15 minutes at 4°C. The cultured fibroblasts were washed and lysed. Protein levels were then quantified using the BCA protein assay kit. Protein samples were electrophoresed in SDS-PAGE to separate protein bands. The protein was transferred from the gel to a PVDF membrane and blocked with 5% skimmed milk for 2 hours. PVDF membranes were incubated with polyclonal rabbit antibodies specific for NLRP3, caspase-1, ASC, TGF-β1, VEGF and TIMP-1 overnight at 4°C. The next day, the membrane was incubated with the secondary antibody for 2 hours. The bands were visualized using the ECL method, and ImageJ software was used to quantify the total gray value (average gray value × gray value area) of protein band to calculate the relative value of target protein.

ELISA

Peripheral serum of rats and culture supernatant of cells were respectively collected and centrifuged at 10,000 rpm for 20 min at 4°C, after which the levels of IL-1β and IL-18 were measured by ELISA kits. All steps were performed according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 Software (San Diego, CA, USA). Data were represented as mean ± standard deviation. Group comparisons were assessed with the one-way ANOVA or student's t-test for comparison of multiple columns. $P < 0.05$ (two-tailed) was considered statistically significant.

Results

Synovial fibrosis and hypoxia were found in MIA-induced KOA model rats

The chemical structure of IMP is shown in (Fig. 1A). To elucidate the role of Synovial fibrosis and hypoxia and associated regulatory genes in synovial fibrosis, we examined mRNA expression levels of HIF-1α and fibrogenic factors, TGF-β1, TIMP1 and VEGF by real-time PCR. Protein expression levels were measured by Western blotting. The upregulation of HIF-1α in pathological conditions suggested that the synovial membrane was under hypoxia conditions with synovial fibrosis induced by MIA in rats. HIF-1α protein and mRNA levels were markedly elevated in KOA synovial membranes (Fig. 1B). Then we investigated the mRNA and protein expression level of TGF-β1, TIMP1 and VEGF in fibrotic synovium were all upregulated in pathological conditions induced by MIA in rats (Fig. 1C). Representative synovium tissues of each group stained with collagen fiber staining by Sirius red staining or H&E staining (Fig. 1D).

Activation of NLRP3 inflammasome in synovial membranes resulted in synovitis in rats

As synovial fibrosis usually followed by synovitis. The activation of NLRP3 inflammasome is a classic pathway which can promote the expressions of inflammatory factors IL-1β and IL-18. Activation of NLRP3 inflammasome partly contribute to synovitis. Key inflammatory factors of NLRP3 inflammasome-dependent cytokines, IL-1β and IL-18, were measured by ELISA, which were significantly upregulated in the MIA group compared with the NORMAL group (Fig. 2C). Caspase-1 recruited by NLRP3 inflammasome cleaved pro-IL-1β and pro-IL-18 that promoted the maturation of inflammatory cytokines. We then evaluated the mRNA and protein expression of NLRP3 inflammasome in the MIA group. Both mRNA and protein levels were upregulated compared to the NORMAL group (Fig. 2A and B).

IMP inhibited experimental synovitis and synovial fibrosis in rats

IMP, one of the representative components in furanocoumarin, is extracted from Angelica dahurica. In MIA+IMP group, less resident cell hyperplasia, formation of lining cell layers and inflammatory infiltration existed in H&E staining compared with MIA group. In sirius red staining, the collagen fibers were red, the nucleus was green, and other components were yellow. The MIA group showed more collagen fibers than the Control group and IMP ameliorate synovitis and synovial fibrosis (Fig. 1D). After the administration of IMP, the expression of NLRP3, ASC, caspase-1 and caspase-1 p10 were determined by qRT-PCR and Western blotting. All these components related to NLRP3 inflammasome were significantly downregulated in MIA+IMP group when compared with the MIA group both gene and protein level. The production of caspase-1 (p10) was also significantly reduced by IMP compared with MIA group, as well as IL-1β and IL-18 (Fig. 2A-C). Besides, fibrogenic factors TGF-β1, TIMP-1 and VEGF were downregulated after the administration of IMP both in gene and protein level (Fig. 1C). Collectively, the administration of IMP inhibited synovitis, synovial fibrosis and hypoxia pathogenesis in rats.

IMP inhibited LPS-induced upregulation of fibrogenic factors in fibroblasts

We next examined the *in vitro* role of IMP in LPS-stimulated primary cultured fibroblast-like synoviocytes. The upregulated expression of fibrogenic factors suggested their possible involvement in synovial

fibrosis. Treatment of fibroblast-like synoviocytes with LPS (5 μg/mL) significantly upregulated TGF-β1, TIMP-1, and VEGF both in gene and protein level. These fibrogenic factors contribute to synovial fibrosis *in vivo*. After the administration of IMP, all those factors were downregulated significantly (Fig. 3A and B).

IMP inhibited activation of NLRP3 inflammasome in LPS-stimulated fibroblasts

Then we observed significantly upregulated NLRP3, ASC and caspase-1 expression in the LPS group compared with the NORMAL group both in gene and protein level. IMP significantly inhibited the gene and protein expression of NLRP3 inflammasome compared with the LPS group. Production of cleaved caspase-1 and caspase-1 p10 was also confirmed by Western blotting and qRT-PCR. It showed significantly upregulation in the LPS group compared with the NORMAL group and this trend was suppressed by IMP (Fig. 4A and B). The downstream production IL-1α and IL-18 were upregulated after LPS stimulation, and these inflammatory cytokines secreted by fibroblasts were downregulated by IMP (Fig. 4C).

Discussion

Hypoxia, inflammation and fibrosis persistently exist in the pathological progress of KOA [20]. Herein, we demonstrated the therapeutic effects of IMP on KOA. IMP significantly inhibited the expression of HIF-1α gene and protein, activation of NLRP3 inflammasome and downregulated the level of fibrogenic factors in KOA. KOA is characterized by the progressive destruction of articular cartilage and surrounding tissues, especially synovial tissue [21]. In this study, we show for the first time that the IMP intervened with the pathological processes of KOA. The therapeutic effects of IMP may be related to the suppression of inflammation and synovial fibrosis.

Increased HIF-1α is highly involved in the progression of diseases, as well as synovial fibrosis and inflammation. It is well established that low oxygen tension exists in the synovium when KOA happens because of synovial angiogenesis and inflammatory cell infiltration [22]. Hypoxia in microenvironment is mainly marked by the HIF-1α expression. Compared with normal group rats, the expression of HIF-1α and its target genes VEGF and TIMP-1 in synovial tissue were significantly increased in KOA group [23]. We inferred that inhibition of HIF-1α expression could inhibit synovial fibrosis to some extent. In addition, HIF-1α can regulate NLRP3 expression. Under the condition of hypoxia, the expression of hypoxia-inducible factor-1α increased, the expression of NLRP3, Caspase-1and IL-1β also increased [24]. HIF-1α is known to regulate a plethora of human diseases. By regulating NLRP3 (transcript) expression under these conditions, it becomes a key node in linking hypoxia response to pro-inflammatory status. Increased expression of NLRP3 and enzymatic activation of caspase-1 are one of the conditions for the upregulation of IL-1β and IL-18. And these increased inflammatory factors were driven by the NLRP3 inflammasome by caspase-1. We interestedly found that IMP can not only inhibit the expression of HIF-1α in fibroblasts, but also the activation of NLRP3 inflammasome and the expression of downstream IL-1β and IL-18. Our previous research shows that inhibition of HIF-1α can effectively lead to synovial

fibrosis in KOA rats, so we observed three fibrogenic factors (TGF-β1, VEGF and TIMP-1) at the same time.

Synovial fibrosis is a pathological process observed in several musculoskeletal diseases. HIF-1α regulates the expression of genes and proteins related to angiogenic growth factors, such as the expression of VEGF and TGF-β1 in RA [25]. TGF-β1 is one of the major indicators of synovial fibrosis that activate myoblasts, promote ECM gene expression, and inhibit ECM degradation [26]. However, the triggering mechanism of synovial fibrosis in the knee joint is not completely clear. Ko et al. found that TIMP1 was up-regulated both in OA fibroblasts stimulated with TGF-β1 and in mice with TGF-β-induced fibrosis [27]. TIMP-1 is an inhibitor of matrix metalloproteinases. We found that TIMP-1 is also elevated in the synovium of human end-stage OA patients [5]. TIMP-1 is induced by TGF-β, but is usually considered as an accelerator of fibrosis development not itself involved in inducing fibrosis [28]. A recent study showed that the articular capsule of the fixed knee joint was in a state of hypoxia, VEGF was up-regulated at the mRNA and protein levels after immobilization. The decoy ODN transfected with HIF-1 successfully suppressed the transcriptional activation of HIF-1. Expression of VEGF was subsequently suppressed [29]. In RA, hypoxia is caused by increasing metabolic requirements for white blood cells to enter RA joints, which can cause HIF-1α to accumulate in the cytoplasm and induce expression of RA synovial tissue fibroblast and secrete VEGF. Positive feedback regulation of the HIF-1α and VEGF pathways can trigger angiogenesis during hypoxia. In addition, the levels of VEGF and HIF-1α in synovial tissue were positively correlated with microvascular density [30]. In this study, IMP decreased TGF-β1, TIMP1 and mRNA and protein levels *in vitro* and *in vivo*. Therefore, IMP may inhibit synovial fibrosis by inhibiting the expression of fibrogenic factors.

IMP is one of the furanocoumarin derivatives and exists in many Chinese herbal medicines with anti-tumor, antibacterial, cardiovascular, anti-inflammatory activities. In our previous studies, we found that synovial fibrosis is highly correlated with the activation of HIF-1α and NLRP3 inflammasome. These results indicate that IMP could improve synovitis and synovial fibrosis by inhibiting HIF-1α/NLRP3 inflammasome signaling.

Conclusions

In summary, IMP can improve synovial hypoxia and synovitis, and thus improved MIA-induced synovial fibrosis in KOA rats. It can reduce the release of inflammatory mediators and up-regulate fibrosis markers induced by MIA or LPS by inhibiting the activation of NLRP3 inflammasome. Taken together, IMP may be a potentially effective therapy for KOA, especially for synovial fibrosis. Further studies are required to determine the signal pathway involved in HIF-1α/NLRP3 inflammasome activation/fibrosis. Its mechanism provides new ideas and means for understanding and treating KOA-related synovitis and synovial fibrosis.

Abbreviations

CMC-Na: Carboxymethylcellulose sodium; ECM: extracellular matrix; FBD: fetal bovine serum; H&E: hematoxylin and eosin; HIF: hypoxia-induced factor; IL: interleukin; IMP: imperatorin; KOA: knee osteoarthritis; LPS: lipopolysaccharide; MIA: monosodium iodoacetate; TIMP: tissue matrix metalloproteinase; VEGF: vascular endothelial growth factor.

Declarations

Acknowledgments

We are grateful to the considerable contributions of all coauthors.

Authors' contributions

Haosheng Zhang, Liang Ding and Peimin Wang participated in the design of the study. Haosheng Zhang and Xiaoqing Shi performed the experiments, analyzed the data and wrote the manuscript. Liang Ding, Xiaoqing Shi, Wei Mei, Zhengquan Huang, Li Zhang, Xiaochen Li and Li Zhang performed the experiments and analyzed the data. Peimin Wang considerably revised this manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal protocols were approved by the Animal Care and Use Committee of the Nanjing University of Chinese Medicine. All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Consent for publication

All authors have consented the manuscript been published.

Competing interests

All authors declare no conflicts of interest.

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Tables

Table 1 Primer sequences

mRNA	Forward	Reverse
HIF-1α	5’-CCGCAACTGCCACCACTGATG-3’	5’-TGAGGCTGTCCGACTGTGAGTAC -3’
NLRP3	5’-GAGCTGGACCTCAGTGACAATGC-3’	5’-ACCAATGCGAGATCCTGACAACAC -3’
ASC	5’-AGAGTCTGGAGCTGTGGCTACTG-3’	5’-ATGAGTGCTTGCCTGTGTTGGTC-3’
Caspase-1	5’-ATGGCCGACAAGGTCCTGAGG-3’	5’-GTGACATGATCGCACAGGTCTCG-3’
TGF-β1	5’- GCAACAATTCCTGGCGTTACCTTG-3’,	5’-TGTATTCCGTCTCCTTGGTTCAGC-3’
TIMP1	5’-GCGTTCTGCAACTCGGACCTG-3’	5’-GTGTAGGCGAACCGGATATCTGTG-3’
VEGF	5’-AGCGTTCACTGTGAGCCTTGTTC-3’	5’-CCGCCTTGGCTTGTCACATCTG-3’.

Figures

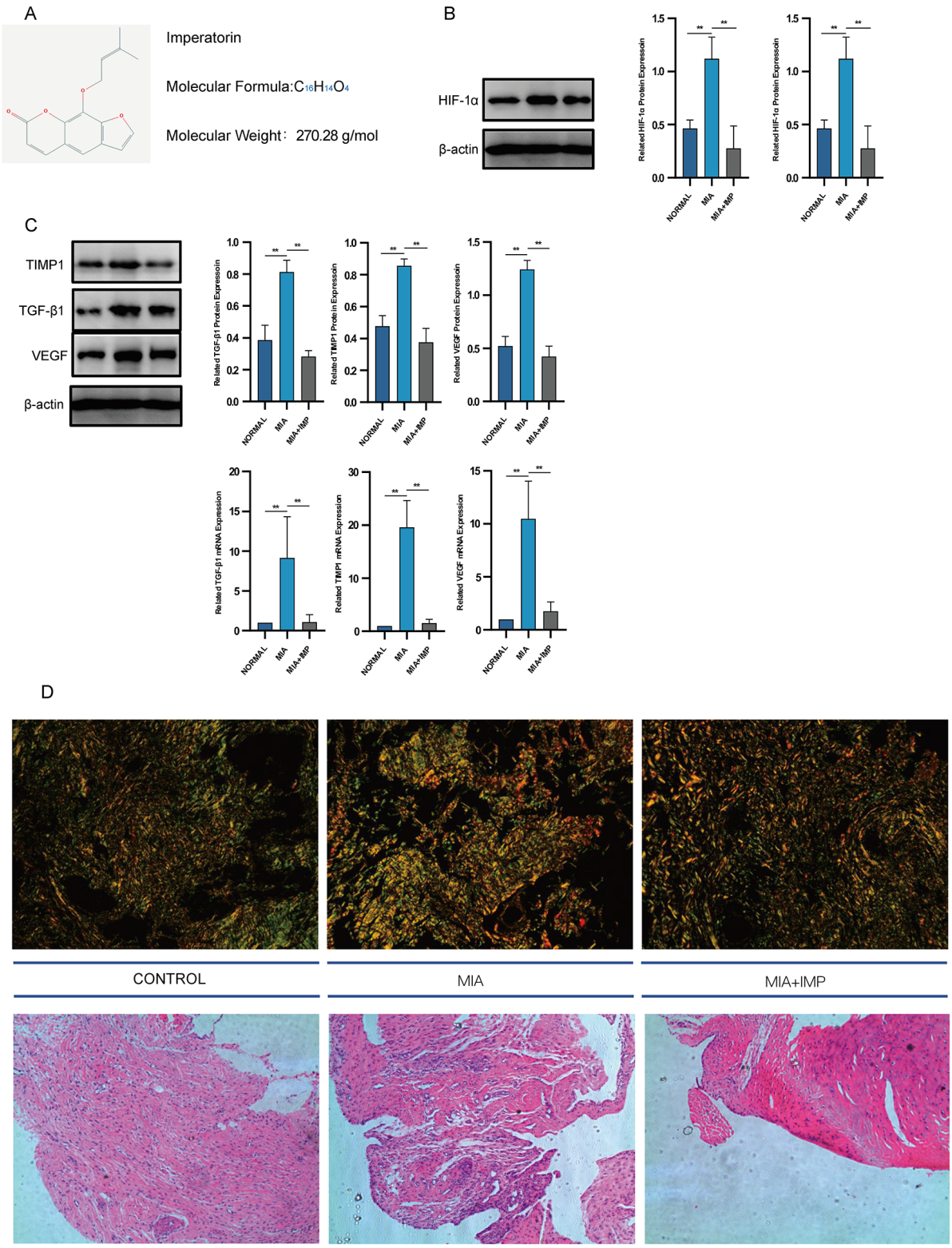

Figure 1

Fibrogenic and hypoxia markers were upregulated in MIA-induced KOA rats and was downregulated by IMP. (A) Chemical structure of imperatorin. (B) mRNA and protein levels of HIF-1α in Normal, MIA-induced KOA rats and KOA rats treated with IMP determined by qRT-PCR and Western blotting. (C) mRNA and protein levels of TGF-β1, TIMP1 and VEGF in Normal, MIA-induced KOA rats and KOA rats treated with IMP determined by qRT-PCR and Western blotting. (D) Representative synovium tissues of each group

undergoing collagen fiber staining by Sirius red staining or H&E staining, 200X, scale bar = 100 μm. Values are represented as mean ± SEM (*p < 0.05, **p < 0.01).

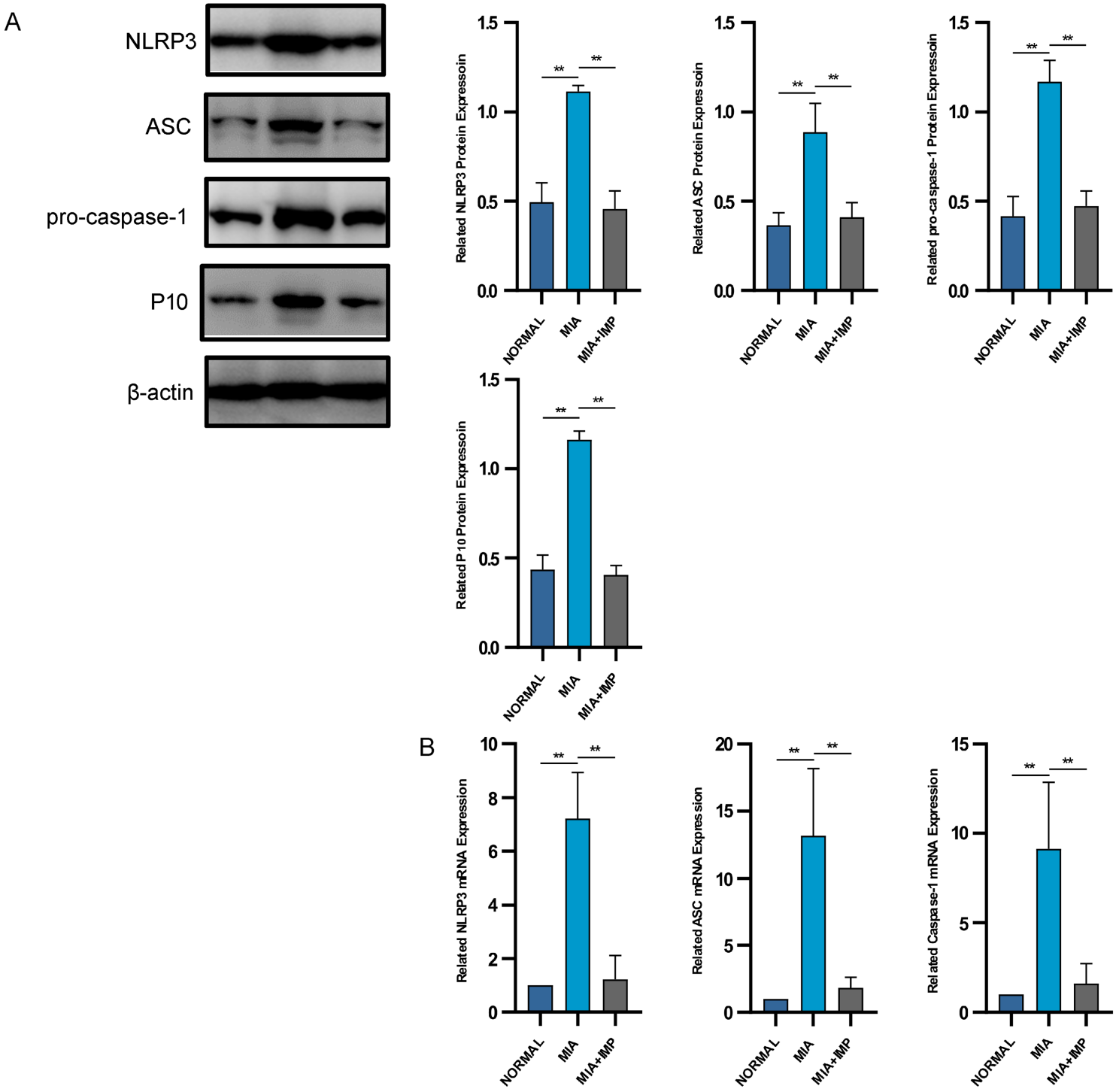

Figure 2

NLRP3 inflammasome was activated in MIA-induced KOA rats and was partly inhibited by IMP. (A) Expression of NLRP3, ASC, pro-caspase-1 and P10 determined by Western blotting. The upregulation of NLRP3 inflammasome protein expression was inhibited by IMP. (B) mRNA levels were quantified by qRT-PCR in fibroblasts treated with or without LPS (5 μg/mL) or treated with LPS (5 μg/mL) and IMP. The

upregulation of NLRP3 inflammasome mRNA expression was inhibited by IMP. Values are represented as mean ± SEM (*p < 0.05, **p < 0.01).

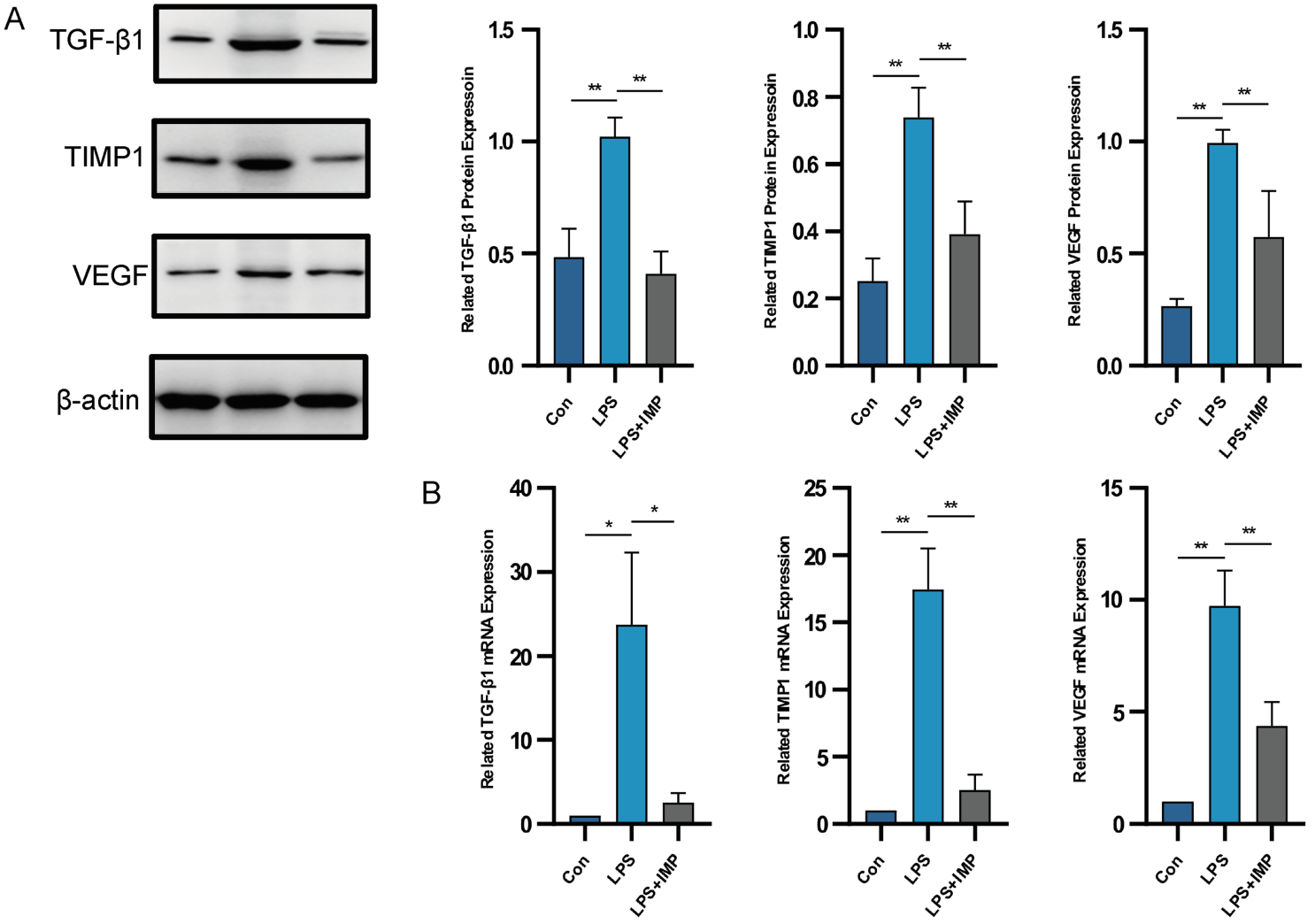

Figure 3

Fibrogenic markers induced by LPS in fibroblasts was upregulated and was downregulated by IMP. (A) Expression of TGF-β1, TIMP1 and VEGF determined by Western blotting. The protein expressions of these fibrogenic markers were inhibited by IMP. (B) mRNA levels were quantified by qRT-PCR in fibroblasts treated with or without LPS (5 μg/mL) or treated with LPS (5 μg/mL) and IMP (50 μM). The mRNA expressions of these fibrogenic markers were inhibited by IMP. Values are represented as mean ± SEM (*p < 0.05, **p < 0.01).

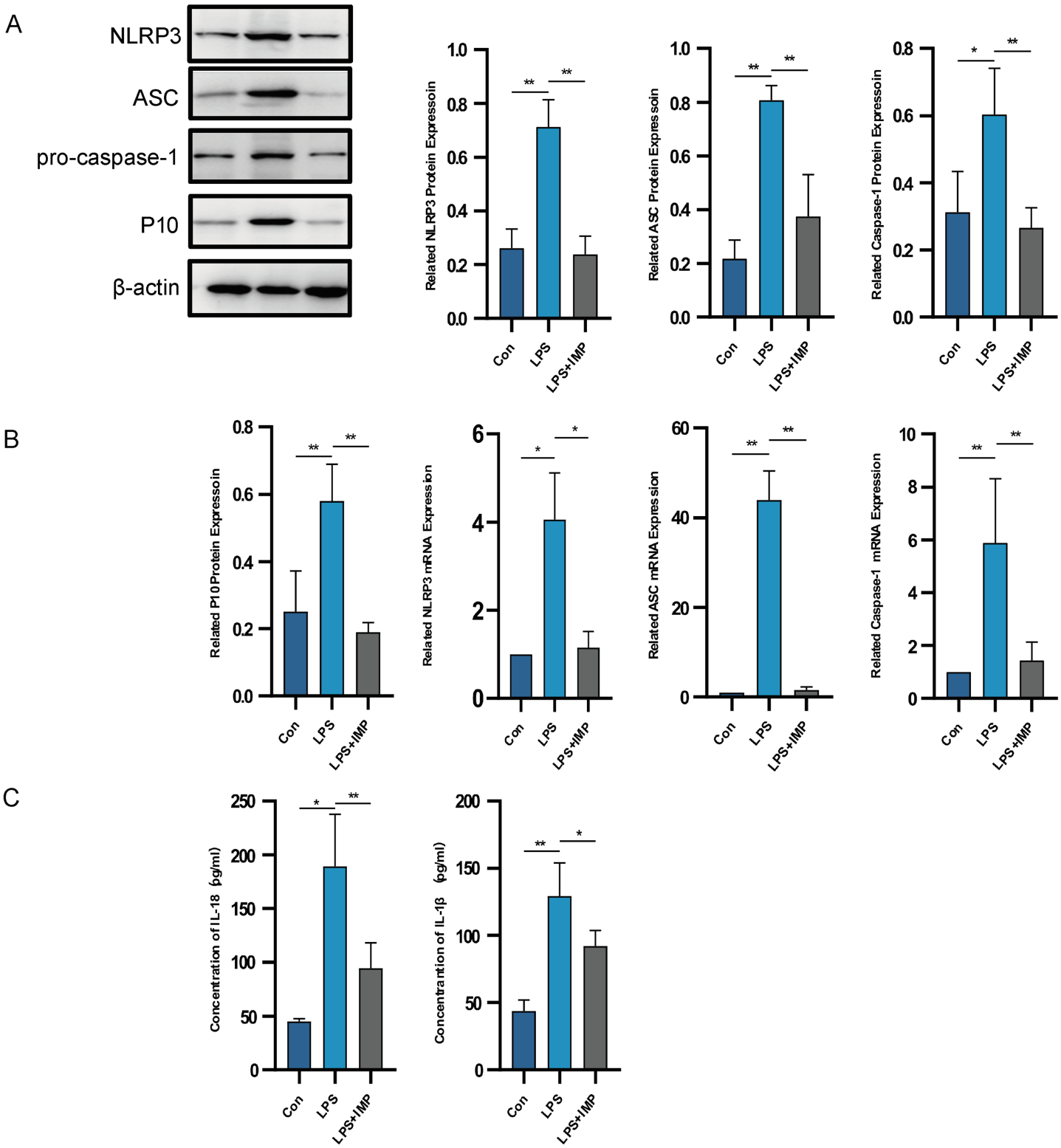

Figure 4

NLRP3 inflammasome was activated by LPS in fibroblasts and was partly inhibited by IMP. (A) Expression of NLRP3, ASC, pro-caspase-1 and P10 determined by Western blotting. The protein expression levels were inhibited by IMP compared to those in the LPS group. (B) The mRNA levels were quantified by qRT-PCR in fibroblasts treated with or without LPS (5 μg/mL) or treated with LPS (5 μg/mL) and IMP (50 μM). The mRNA expression levels were inhibited by IMP compared to those in the LPS

group. Values are represented as mean ± SEM (*p < 0.05, **p < 0.01). (C) Inflammation cytokines IL-1β and IL-18 were measured by ELISA. Values are represented as mean ± SEM (*p < 0.05, **p < 0.01).