International Immunopharmacology 74 (2019) 105575

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

The selective NLRP3-inflammasome inhibitor MCC950 reduces myocardial fibrosis and improves cardiac remodeling in a mouse model of myocardial infarction

Rifeng Gao[a,1], Huairui Shi[b,1], Suchi Chang[b], Yang Gao[b], Xiao Li[b], Chunyu Lv[a], Heng Yang[a], Haiyan Xiang[a], Juesheng Yang[a], Lei Xu[b,**], Yanhua Tang[a,*]

[a] Department of Cardiovascular Surgery, The Second Affiliated Hospital of Nanchang University, Nanchang University, Nanchang, Jiangxi Province, China
[b] Department of Cardiology, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai, China

ARTICLE INFO

Keywords:
NLRP3
MCC950
Fibrosis
Myocardial infarction
Cardiac remodeling

ABSTRACT

Background/Aims.

Early inflammatory responses after myocardial infarction (MI) are likely to increase myocardial fibrosis and subsequent cardiac remodeling. MCC950, a specific NLRP3 inhibitor, was previously found to effectively inhibit the release of inflammatory factors IL-18 and IL-1β. In this study, we evaluated the effect of MCC950, as a potential new treatment strategy for MI, on myocardial fibrosis and cardiac remodeling using an experimental mouse model.

Methods: Male C57BL/6 mice were subjected to left coronary artery ligation to induce MI and then treated with MCC950 (10 mg/kg) or PBS for 14 days. After 30 days, echocardiography was performed to assess cardiac function and myocardial fibrosis was evaluated using H&E- and Masson's Trichrome-stained sections. Myocardial expression of inflammatory factors and fibrosis markers was analyzed by western blotting, immunofluorescence, ELISA, and real-time quantitative PCR.

Results: The ejection fraction in the 10 mg/kg group (40.7 ± 4.2%; $N = 6$, $p = 0.0029$) was statistically preserved compared to that in the control group (14.0 ± 4.4%). Myocardial fibrosis was also reduced in MCC950-treated animals (MCC950, 23.2 ± 3.0 vs PBS, 36.2 ± 3.7; $p < 0.05$). Moreover, myocardial NLRP3, cleaved IL-1β, and IL-18 levels were reduced in MCC950-treated animals. H&E and molecular examination revealed decreases in inflammatory cell infiltration and inflammatory factor expression in the heart. In vitro, MCC950 inhibited NLRP3, reduced caspase-1 activity, and further downregulated IL-1β and IL-18.

Conclusion: MCC950, as a specific NLRP3 inhibitor, can alleviate fibrosis and improve cardiac function in a mouse model by suppressing early inflammatory responses post-MI.

1. Introduction

Myocardial infarction (MI) is a major cause of morbidity and mortality worldwide [1]. Although reperfusion strategies are successful in limiting injury to the heart, reducing infarct size, and improving overall prognosis, patients' risk of heart failure increase both over the short-term and long-term post MI [2]. MI-induced injury causes acute necrosis of cardiomyocytes in the affected area of the heart, concomitant with the generation of a reparative fibrotic scar, which could be one natural protective mechanism to prevents ventricular wall rupture. However, in the long-term, excessive interstitial fibrosis in the heart might lead to progressively worse cardiac function and stiffening of the wall and septal [3]. Another factor affecting the cardiac function and remodeling is the inflammatory responses post-MI. It is known that inflammatory responses can further aggravate cardiac remodeling and myocardial injury [4]. Further, inflammasome formation in the mouse heart during MI can cause the additional loss of functional myocardium, finally leading to heart failure [5]. Inflammatory bodies are large, multi-cytoplasmic protein complexes formed in response to damage and pathogen-associated molecular patterns that, serve as platforms for

* Correspondence to: Y. Tang, Department of Cardiovascular Surgery, The Second Affiliated Hospital of Nanchang University, No. 1 Minde Road, 330006 Nanchang, Jiangxi Province, China.
** Corresponding author.
E-mail addresses: xu.lei2@zs-hospitai.sh.cn (L. Xu), tyh6565@163.com (Y. Tang).
[1] Rifeng Gao and Huairui Shi contribute equally to this manuscript.

https://doi.org/10.1016/j.intimp.2019.04.022
Received 20 December 2018; Received in revised form 8 April 2019; Accepted 9 April 2019
Available online 09 July 2019

caspase-1 activation [6]. Most inflammasomes contain a protein of the NLR family of proteins (NLRP1, NLRP3, NLRP6, NLRP7, NLRP12 or NLRC4), cysteine protease caspase-1, and apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) [7]. The NLRP3 inflammasome plays a key role in identifying danger signals and inducing sterile inflammatory responses in response to MI. When activated, the pyrin domain of NLRP3 interacts with ASC, promoting the recruitment of caspase-1 by the ASC C-terminal domain and leading to NLRP3 inflammasome assembly with subsequent caspase-1 activation, which helps to process pro-IL-18 and pro-IL-1β into their biologically active and mature forms [7,9]. Hence, NLRP3 represents an integral part of the innate immune system, providing fine regulation of caspase 1 activation and the facilitating the production and secretion of influential pro-inflammatory cytokines [10]. However, NLRP3-IL-1β signaling underlies many diseases, including cancer, gout, osteoarthritis, periodic auto-inflammatory syndromes and cardiac dysfunction [11,12]. MI-induced myocardial injury stimulates NLRP3 inflammasome assembly, which promotes the secretion of inflammatory factors such as IL-1β and IL-18 and thus aggravates myocardial damage leading to systolic dysfunction [13,15].

Several NLRP3 inhibitors have been discovered recently, including MCC950, β-hydroxybutyrate type I interferon (IFN) and interferon-β. It is known that MCC950, which contains a diarylsulfonamide group, not only inhibits the caspase-1 dependent production of IL-1β, but also inhibits the activation of NLRP3 inflammatory bodies, thereby inhibiting the secretion of IL-1β and IL-18 and reducing the severity of experimental autoimmune encephalomyelitis and cryopyrin-associated periodic syndromes in mice [16]. However, MCC950 suppresses caspase-1 activation, caspase-1 mediated uptake and production, and secretion of ILs without affecting the activation of NLRC4 or AIM2 inflammasomes [17,18].

MCC950, the most specific and well-characterized NLRP3 inhibitor to date, was proven to play a major role in a variety of inflammatory diseases in which NLRP3 is involved. Studies suggest that MCC950 can protect cardiac function and reduce infarct size in a pig animal ischemia reperfusion (I/R) model [19]. However, the long-term myocardial protective effect of MCC950 and its mechanism of action in the heart are still unclear. In particular, the effect of this compound on cardiac outcomes after MI is unclear. Hence, we hypothesized that MCC950 might be effective in reducing myocardial fibrosis and improving cardiac function after MI by inhibiting the expression of NLRP3 inflammatory corpuscles in fibroblasts in a mouse model of MI.

2. Materials and methods

2.1. Mouse model of MI

Male C57/BL6 mice weighing 25–30 g were maintained on a regular 12-h light/dark cycle with free access to standard mouse chow (The Second Affiliated Hospital of Nanchang University, Nanchang, China) and water. The mice were assigned to either the MI group (surgery to induce MI by occlusion of the left coronary artery) or sham group (same operation but no left coronary artery occlusion). Surgery was conducted on mice anesthetized via 2% isoflurane inhalation. MI mice were then randomized to receive intraperitoneal injection of 10 mg/kg MCC950 (S7809; Selleck Chemicals, Houston, TX, USA) or phosphate buffered saline (PBS), which served as the comparison group, three times per week for 2 weeks. Heart tissue on day 3, 7, 14 and 30 was analyzed by immunohistochemistry and molecular experiments.

All experiments were approved by the Animal Ethics Committee of the Second Affiliated Hospital of Nanchang University and were conducted in compliance with the ARRIVE guidelines. Mice were handled in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Echocardiographic assessment

To evaluate cardiac geometry and function at 30 days after MI, transthoracic echocardiography was performed using a 30-MHz high-frequency scan head (Vevo770; Visual Sonics Inc. Toronto, Canada). MI-mode imaging was conducted using the left ventricular (LV) parasternal long-axis view and all. Data were averaged based on the measurements of at least six cardiac cycles including records of LV end-systolic diameter, end-diasolic diameter, ejection fraction and fractional shortening.

2.3. Histological analysis

Tissues were fixed in 4% paraformaldehyde, sectioned, and processed for hematoxylin-eosin and Masson's trichrome immunohistochemical staining. Masson trichrome staining was performed according to a previous report [20]. For immunohistochemical analysis, the antibodies used were anti-mouse CD68 (ab31630; Abcam), Ly6G (ab25377; Abcam) and CD4 (ab183685; Abcam).

2.4. In vitro cell experiments

Primary cardiac fibroblasts were isolated from 1 to 3-day-old C57/BL6 mice purchased from the Changzhou Cavans Experimental Animal Co., Ltd. Mouse cardiac fibroblasts were cultured as described previously [21,22]. Briefly, heart cells of 3-day-old C57/BL6 mice were isolated and cultured with 0.2% collagenase type II (Worthington, USA). Cardiac fibroblasts were purified by pre-plating the cells on cell culture dishes for 60 min. Subsequently, the fibroblasts were cultured in 4.5 g/L of D-glucose DMEM (Life Technologies, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS, Hyclone, USA) and 1% penicillin/streptomycin (Hyclone, USA) with standard culture conditions (37 °C, with 5% CO_2). Cells were passaged at 70–80% confluence. The cells obtained from the second passage were used for the experiments.

2.5. Cell immunofluorescence

The fibroblasts were seeded on a cover glass. After fixing with 4% paraformaldehyde at room temperature for 10 min, cells were treated with 0.1% triton X-100 for 10 min and blocked with 5% BSA at room temperature for 30 min, and then incubated with primary antibodies (NLRP3, ab4207, Abcam, Cambridge, MA, USA; Vimentin, ab195877, Abcam, Cambridge, MA, USA; CD31, ab28364, Abcam, Cambridge, MA, USA; Monoclonal Anti-α-Actinin, A7811MSDS, Sigma-Aldrich, St. Louis, MO 63178, USA) overnight at 4 °C. The fibroblasts were then incubated with secondary fluorescent antibodies (FITC conjugated goat anti-rabbit IgG, GB22303, Servicebio, Wuhan, China; fluorescein isothiocyanate labeled goat anti-mouse IgG, GB22301, Servicebio, Wuhan, China) for 1 h in the dark at room temperature. Then, the cells were incubated with DAPI (G1012, Servicebio, Wuhan, China) for 10 min in the dark at room temperature. These samples were observed using a confocal laser scanning microscope.

2.6. Real-time quantitative PCR

Total cellular RNA was purified from cardiac tissues and cardiac fibroblasts using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA samples were subsequently reverse-transcribed to cDNA (TaKaRa Bio, Osaka, Japan). The following conditions were used for thermal cycling: denaturation at 95 °C for 30 s, 40 cycles of priming at 95 °C for 5 s, and elongation at 60 °C for 20s. Primer pairs were used (Sangon Biotech, Shanghai) for each target gene, as summarized in Supplementary data Table 1. The transcript levels were normalized to those of GAPDH. Data were analyzed using the comparative CT(2-ΔΔCT) method as previously described [23].

2.7. ELISA assays

IL-1β concentrations were measured using standard ELISA kits (RK00006; ABclonal, Biotechnology, Wuhan, China). Data are presented for individual cytokines, as well as for the ratios between the levels of different cytokines.

2.8. Western blot assay

Cardiac tissues and fibroblasts were harvested in RIPA lysis buffer with 1 mM phenylmethanesulfonyl fluoride. Protein concentrations were determined using a BCA protein assay kit (WB0101; Biotech Well, Shanghai, China); samples were separated by 10% and 15% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (all from Biotech Well). The membranes were blocked with 5% bovine serum albumin in TBST for 1.5 h and incubated with primary antibodies against NLRP3 (15101S; Cell Signaling Technology, Danvers, MA, USA; 1:1000), caspase-1 (ab108362; Abcam, Cambridge, MA, USA; 1:1000), IL-18 (ab71495; Abcam; 1:1000), IL-1β (31202; Cell Signaling Technology; 1:1000), collagen-I (ab6038; Abcam; 1:1000), collagen-III (ab7778; Abcam; 1:1000), Vimentin (ab195877, Abcam; 1:1000), CD31 (ab28364, Abcam; 1:1000), Monoclonal Anti-α-Actinin (A7811MSDS, Sigma-Aldrich, St. Louis, MO 63178, USA; 1:1000), α-SMA (19245; Cell Signaling Technology; 1:1000), β-actin (4970S; Cell Signaling Technology; 1:4000), and GAPDH (5174S; Cell Signaling Technology; 1:4000) overnight at 4 °C and then with horseradish peroxidase-conjugated secondary antibodies for 1.5 h at room temperature. Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA) was used for detection. Images of the gel were captured using the Image Quant LAS 4000 mini (GE Healthcare, Barrington, IL, USA).

2.9. Statistical analyses

Results are expressed as the mean ± standard error of the mean. Statistical analyses were conducted using GraphPad Prism 5.01 and SPSS 18.0 (SPSS Inc., Chicago, IL). The normality of data distribution was tested using the Kolmogorov-Smirnov test. One-way ANOVA with a post-hoc Holm-Sidak test (if $p < 0.05$ with no significant variance in homogeneity) was used for multiple-group comparisons.

3. Result

3.1. MCC950 helps to preserve cardiac function after MI

At 30 days post MI (representative echocardiograms illustrated in Fig. 1A), echocardiographic parameters were significantly restored in mice treated with MCC950 at 10 mg/kg, compared to those in PBS-treated controls (LVEF: 40.7 ± 4.2 vs 14.0 ± 4.4, Fig. 1B; LVFS: 20.1 ± 2.5 vs 9.5 ± 3.5, Fig. 1C; LVEDD: 4.6 ± 0.1 vs 5.5 ± 0.3, Fig. 1D; LVESD: 3.7 ± 0.2 vs 5.1 ± 0.4, Fig. 1E; all p values < 0.05).

3.2. MCC950 treatment reduces myocardial fibrosis in vivo

At 30 days post MI, Masson trichrome staining (representative images in Fig. 2A) revealed significantly less pronounced fibrosis in MCC950-treated mice than in PBS-treated controls (11.1 ± 2.2% vs 20.6 ± 2.2%, Fig. 2B). Furthermore, the mRNA and protein expression levels of collagen-I, collagen-III, and α-SMA were markedly reduced in MCC950-treated mice (Fig. 2C and D). Taken together, these findings demonstrate that MCC950 reduces post-MI myocardial fibrosis in vivo.

3.3. MCC950 reduces the infiltration of inflammatory cells in the heart in vivo

To determine whether the MCC950-mediated decrease in inflammation translates into reduced cardiac fibrosis, we evaluated the expression of factors known to promote myocardial collagenization. The protein expression levels of NLRP3 (Fig. 3A and B), cleaved IL-1β (Fig. 3C and D), and IL-18 (Fig. 3E and F) increased 3 days after MI and decreased gradually thereafter; all three proteins exhibited a comparable trend. MCC950 treatment not only reduced NLRP3 expression, but also reduced the expression of cleaved IL-1β and IL-18 (Fig. 3G and H). ELISA confirmed that serum IL-1β levels were significantly lower in MCC950-treated mice than in PBS-treated controls (Fig. 3I). Hematoxylin–eosin staining revealed that MCC950-treated mice had reduced inflammatory cell infiltration (Fig. 3J and K). Furthermore, immunohistochemical analysis of the hearts after MI showed the main infiltrating inflammatory cells were $CD68^+$ macrophages and $Ly6G^+$ neutrophils. The content of $CD68^+$ macrophages and $Ly6G^+$ neutrophils in infiltrating myocardium decreased after MCC950 treatment (Supplemental Fig. 1). Given the mechanistic role of cleaved IL-1β and IL-18 in promoting fibrosis, these findings confirm that NLRP3 inhibition by MCC950 reduces myocardial fibrosis at least partly by suppressing the release of cleaved IL-1β and IL-18.

3.4. MCC950 can alleviate hypoxic damage in cardiac fibroblasts

Whereas extremely low levels of IL-1β and almost no NLRP3 were detected in fibroblasts incubated under normal conditions, the expression of these markers were significantly higher after 18 h of incubation under hypoxic conditions (Fig. 4A and B), suggesting that hypoxia induces NLRP3 and IL-1β expression in cardiac fibroblasts. As these effects were most pronounced after 18 h of incubation, subsequent experiments with cardiac fibroblasts were performed for this duration.

To clarify the mechanism underlying the effect of MCC950 on cardiac fibrosis, we first performed a CCK-8 assay to evaluate the effects of different concentrations of MCC950 (0.001, 0.01, 0.1, 1, 10, and 100 μmol/L) on the proliferation of fibroblasts. Cell vitality did not differ between MCC950-treated fibroblasts and untreated fibroblasts, indicating that this agent had no significant effect on cardiac fibroblast proliferation (Fig. 4C). Based on western blot analysis, the expression of collagen-I and α-SMA was significantly lower in fibroblasts incubated under hypoxic conditions with 10, 20, or 40 μM MCC950 comapred to levels in untreated fibroblasts incubated under normal or hypoxic conditions (Fig. 4D and E). Thus, results of the in vitro cell culture experiments supported those of the mouse experiments, confirming the beneficial effect of MCC950 as an anti-fibrotic agent.

3.5. MCC950 inhibits NLRP3 signaling and downregulates IL-18 and IL-1β expression in fibroblasts

Fluorescence staining indicated that MCC950 inhibited the expression of NLRP3 inflammatory factors (results in Supplemental Fig. 2). We also found that caspase-1 expression was enhanced in fibroblasts incubated under hypoxic conditions, revealing that the hypoxia-induced increase in NLRP3 expression could directly enhance caspase-1 activity which in turn results in the upregulation of cleaved IL-18 and IL-1β (Fig. 4F and G). However, this effect was attenuated by treatment with 1, 10, 20, or 40 μmol/L of MCC950 (Fig. 4F and G). These results demonstrate that MCC950 counteracts the hypoxia-induced upregulation of NLRP3, resulting in decreased caspase-1 activation and reduced secretion of cleaved IL-1β and IL-18 in mouse cardiac fibroblasts (Fig. 4H).

4. Discussion

MI-induced damage to the heart results in a sterile inflammation that leads to scar formation and infarct healing [26,27]. Appropriate anti-inflammatory therapy reduces myocardial fibrosis and improves cardiac remodeling. In this context, the most important finding of our present study is that by specifically inhibiting NLRP3, MCC950 ameliorates myocardial damage and reduces heart fibrosis, improving

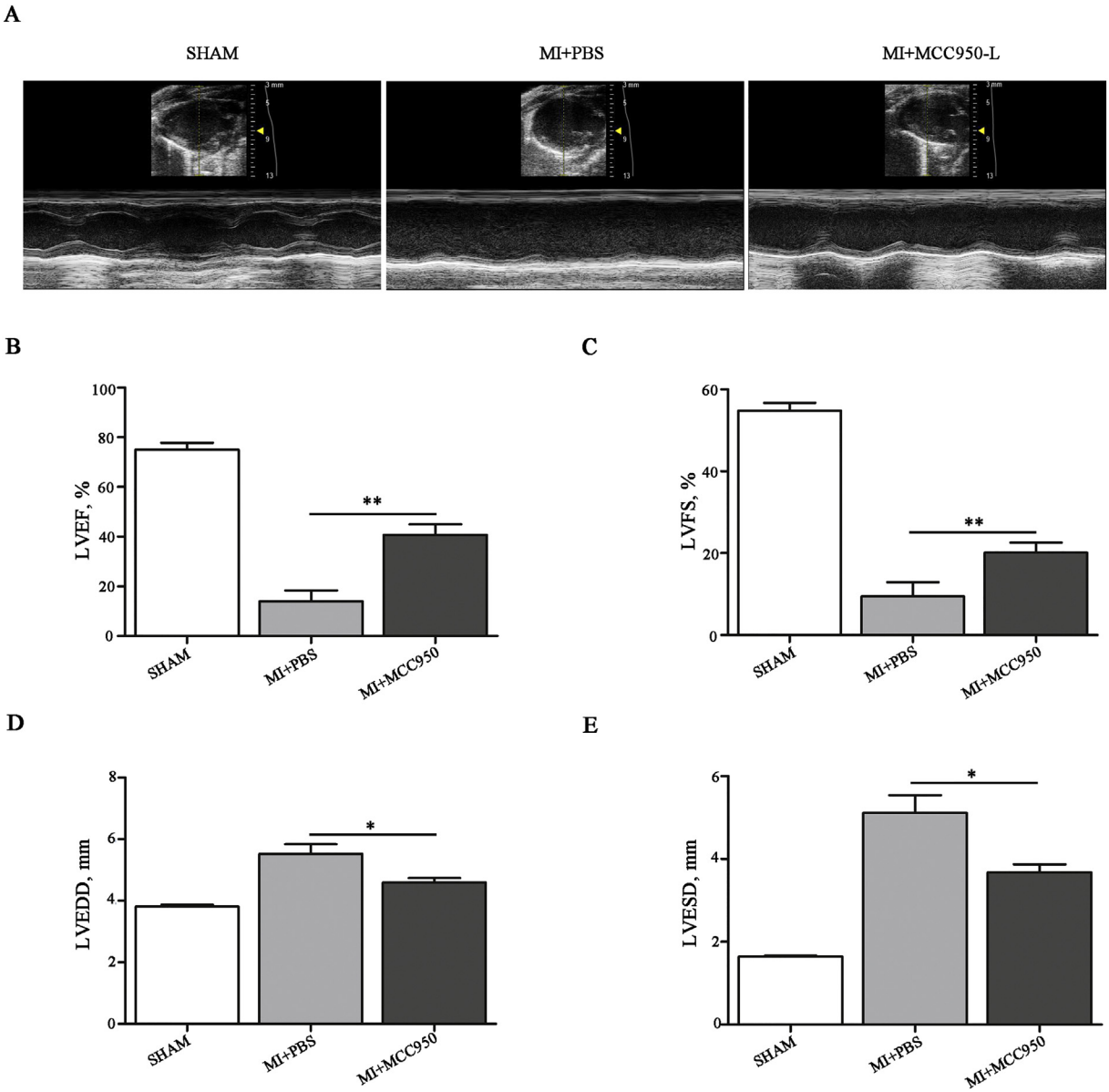

Fig. 1. MCC950 helps preserve cardiac function after MI.
Representative images of echocardiography tracing in the mice with MI treated with MCC950 (10 mg/kg) or PBS in each group at 30 days (A). Left ventricular ejection fraction (LVEF; B). Left ventricular fractional shortening (LVFS; C). Left ventricular end-systolic diameter (LVESD; D). Left ventricular end-diastolic diameter (LVEDD; E). Data are depicted as Mean ± SEM. Differs significantly (one-way ANOVA with a post-hoc Holm-Sidak test, $*P < 0.05$, $**P < 0.01$, compared with the MI + PBS group).

cardiac remodeling, as reflected by the recovery of LV fractional shortening and the ejection fraction at 30 days post-MI. We also discovered that MCC950 blocks activation of the NLRP3 inflammasome in heart tissue and suppresses the recruitment of inflammatory mediators in cardiac fibroblasts.

In our mouse model, levels of the inflammatory factors NLRP3, IL-1β, and IL-18 changed over time, peaking on day 3 or 7, and disappearing by day 14 after MI. The NLRP3 inflammasome is a macromolecular structure that is assembled via the chemotactic recruitment of infiltrating cells, which not only induces the self-cleavage and activation of caspase-1, but also the conversion of pro-IL-1β and pro-IL-18 to IL-1β and IL-18, respectively [28]. IL-1β and IL-18 act as key factors in heart inflammation and play a crucial role in cardiac prognosis after MI. In animals, IL-1β is critically involved in the pathogenesis of cardiac remodeling by regulating extracellular matrix metabolism and thus fibroblast function [29,30]. Specifically, IL-1β activation promotes cell death, myocardial fibrosis, cardiac remodeling, and heart failure after MI [5]. Further, increased plasma levels of IL-18 were reported in MI patients and animal models of MI [31], whereas pretreatment with an IL-18-neutralizing antibody reduced infarct size in mouse MI [32]. Moreover, IL-18 promotes lipopolysaccharide induced myocardial dysfunction and increases the risk of cardiovascular disease in the general population [33,34]. Similar to IL-1β, IL-18 can depress myocardial function and modulate the cardiac remodeling process after injury [35,36]. In the present study, we found that MCC950 intervention can reduce inflammatory infiltration, especially macrophages and neutrophils. We confirmed that MCC950 suppresses serum and myocardial expression of cleaved IL-1β and IL-18, which was corroborated by a decreased fibrotic area and better cardiac function compared to those in PBS-treated mice.

The effects of inflammation induced by myocardial injury might vary with cell type. In cardiomyocytes, the activation of inflammatory

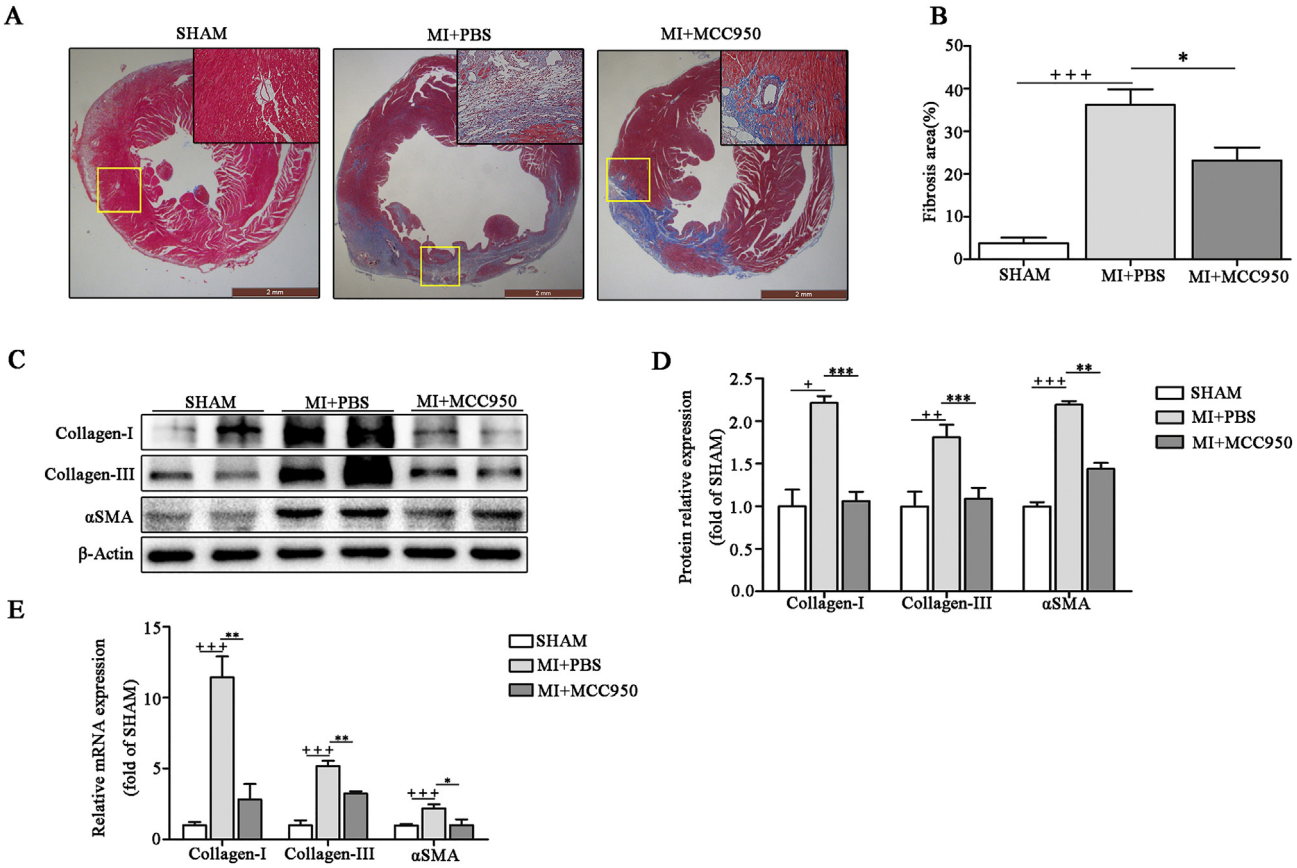

Fig. 2. MCC950 treatment resulted in reduce myocardial fibrosis in vivo.
Masson-trichrome staining of the heart sections from Sham, MI + PBS and MI + MCC950 mice after LAD ligation of the heart (A). The proportional area of blue staining on trichrome-stained sections of the mice hearts (B); Protein expressions of Collagen-I, Collagen-III and αSMA in CFs treatment with PBS or MCC950 were evaluated by Western blot (C and D). MRNA expression of Collagen-I, Collagen-III and αSMA in cardiomyocytes detected by RT-qPCR (E). Data are depicted as Mean ± SEM. Differs significantly (one-way ANOVA with a post-hoc Holm-Sidak test, ($^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$, compared with the Sham group; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with the MI + PBS group). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bodies leads to caspase-1 activation and pyroptosis but is also associated with reduced IL-1β release [37]. In cardiac fibroblasts, inflammasome activation induces the upregulation of active IL-1β, which stimulates collagen synthesis and myofibroblast differentiation, providing the basis for tissue healing. Experimental data suggest that cardiac fibroblasts are also very sensitive to IL-1β and IL-18, which promote myocardial fibrosis and scar healing after MI. Thus, cardiac fibroblasts may be targetted by cardiac IL-1β [38,39]. At the cellular level, IL-1β regulates extracellular matrix proteins in cardiac fibroblasts [40]. Therefore, we used cardiac fibroblasts to study the effect of MCC950 on the release and activation of inflammatory factors (cardiac fibroblasts identified in Supplemental Fig. 3). We found that, under hypoxic conditions, the secretion of inflammatory factors (NLRP3, IL-18, IL-1β) was significantly lower in the MCC950-treated group than in the PBS-treated controls; the same trend was noted for collagen synthesis. We also demonstrated that MCC950 inhibits NLRP3, reduces caspase-1 activity, and inhibits cleaved IL-1β and IL-18 release, thereby reducing collagen secretion in primary cardiac fibroblasts.

MCC950 is one of several diarylsulfonylureadiary sulfonylurea-containing compounds that were identified in 2015 as inhibitors of the NLRP3 inflammasome [16]. In this study, we found that treatment with MCC950 at doses of 10 and 20 mg/kg improved cardiac function in mice after MI. Corresponding protein expression experiments also demonstrated that MCC950, at doses of 10 and 20 mg/kg, inhibites NLRP3 expression in the myocardium (relevant results in Supplemental Fig. 4). In 2017, Gan and van der Heijden et al. found that the use of MCC950 at a dose of 10 mg/kg could inhibit atherosclerotic lesions and reduce the damage caused by hypertension [24,25]. In addition, the results of CCK8 experiments indicated that high-dose MCC950 might be associated with some cytotoxicity. Therefore, we believe that high-dose MCC950 treatment might have drug toxicity, affecting the protective effect on the myocardium in animal experiments, but the specific associated mechanism requires further study. In this experiment, we treated MI mice with lower doses of MCC950. We found that 10 mg/kg of MCC950 could reduce myocardial fibrosis in MI mice and exert a protective effect on cardiac function, which was associated with better LV function 30 days after MI.

To summarize, our present findings indicate that MCC950 inhibits expression of the inflammatory factors IL-1β and IL-18, reduces myocardial fibrosis, and improves cardiac function after MI. Using tissue and cellular studies, we demonstrated that these effects are due to a significant reduction in NLRP3 activation. Our data also support the role of NLRP3 and IL-1β in cardiac fibrosis and heart remodeling. Taken together, our results highlight the potential of MCC950, a selective NLRP3-inflammasome inhibitor, as a novel therapeutic agent for reducing myocardial fibrosis and improving myocardial remodeling after MI. MCC950 treatment could be used as a new strategy for anti-inflammatory treatment for clinical patients with MI in the future.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.04.022.

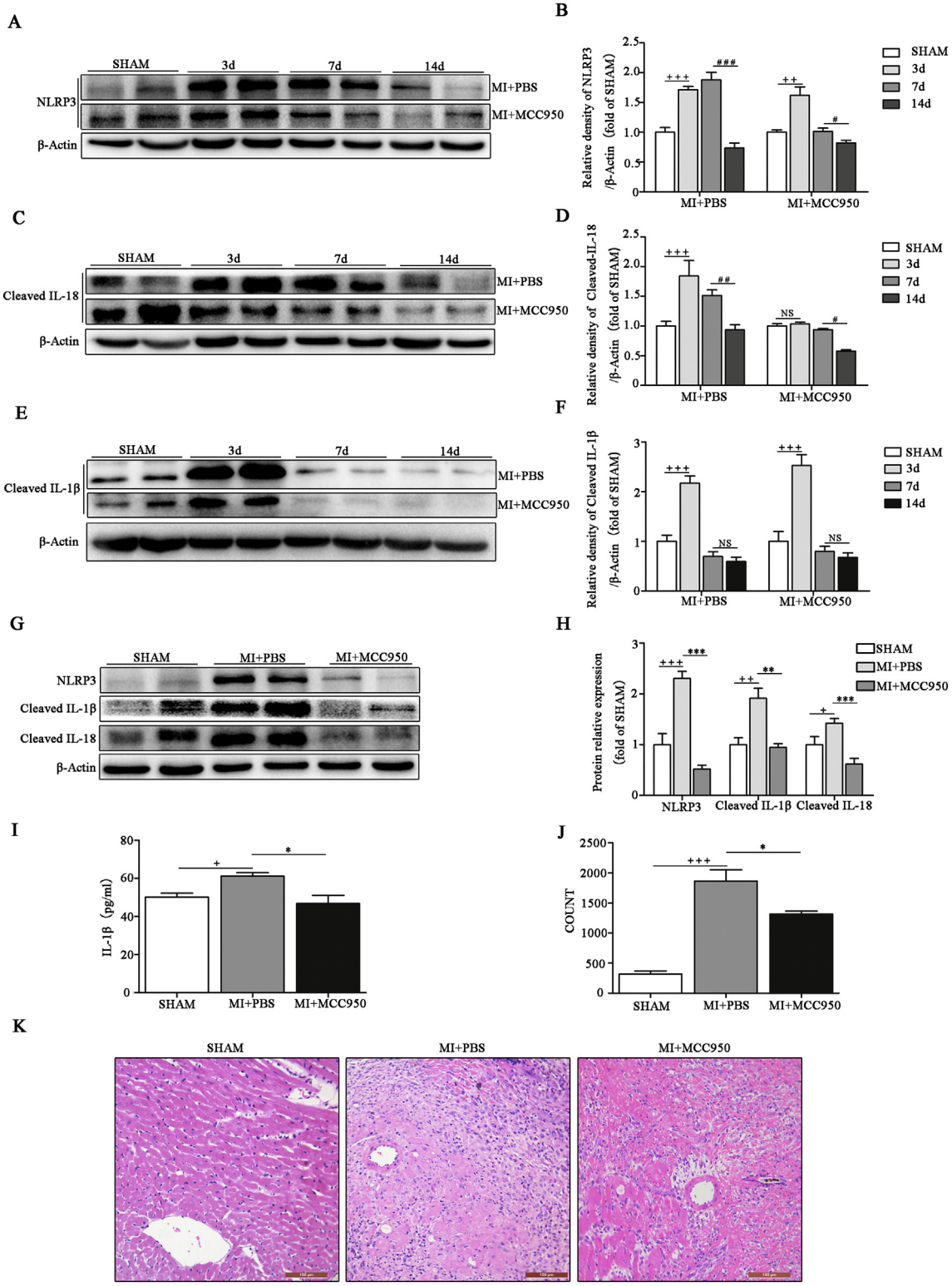

Fig. 3. MCC950 reduces infiltration of inflammatory cells of the heart in vivo. Representative western blot of NLRP3 (A), cleaved IL-1β (C) and cleaved IL-18 levels (E) in mice treated with MCC950 and control animals. Effects of MCC950 on MI model and NLRP3 (B), cleaved IL-1β (D) and IL-18 (F) expression. Addition of MCC950 after MI reduced IL-1β release were evaluated by ELISA (I). Hematoxylin and eosin displaying myocardial inflammation expression from SHAM, MI + PBS and MI + MCC950 (J and K). Data are depicted as Mean ± SEM. Differs significantly (one-way ANOVA with a post-hoc Holm-Sidak test, $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$, NS, not significant, compared with the Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the MI + PBS group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, NS, not significant, compared with the 7d group).

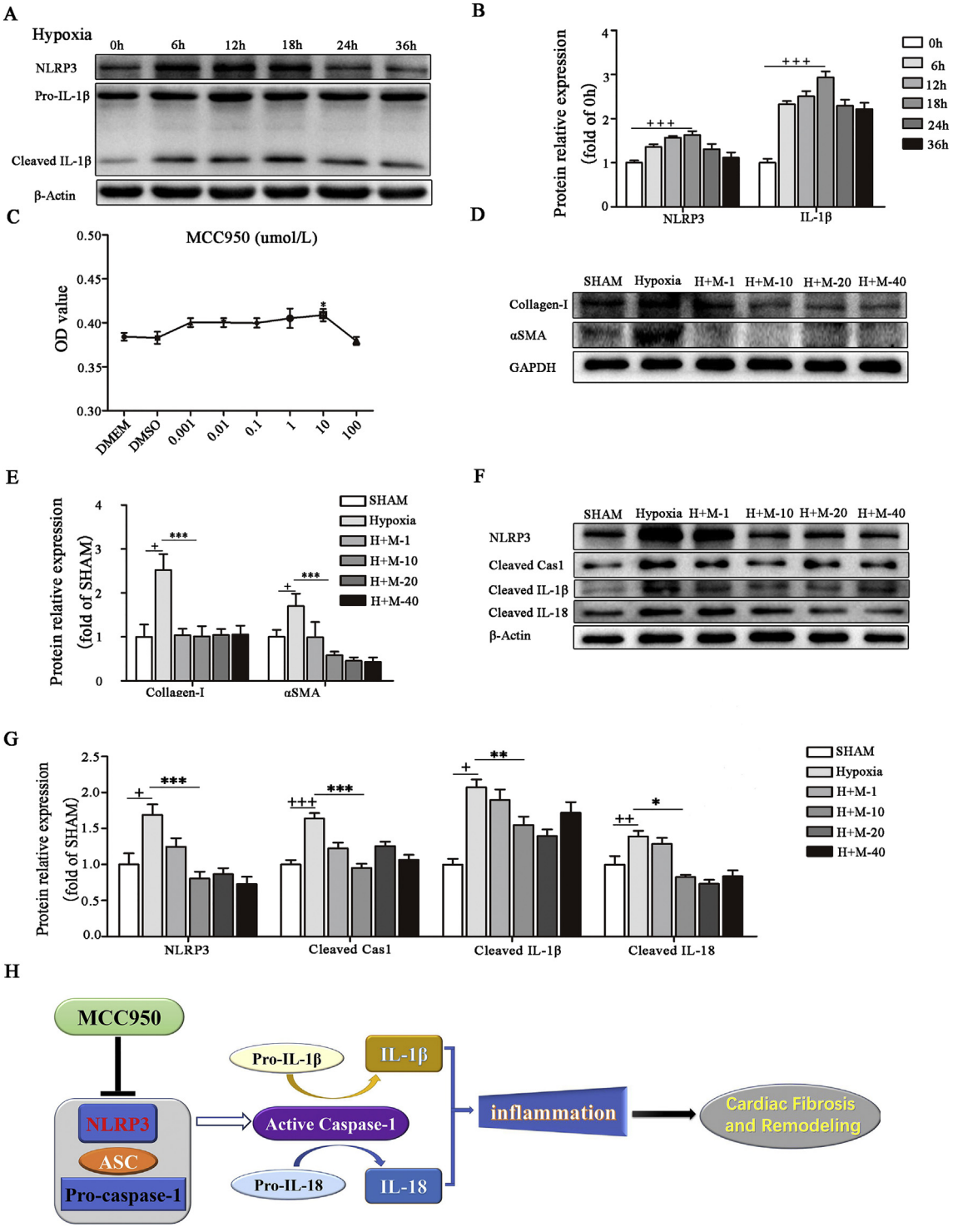

Fig. 4. MCC950 can alleviate the damage caused by hypoxia.
The NLRP3 and IL-1β levels in cardiac fibrobssast supernatant were detected by Western blot in the Hypoxia (0, 6, 12, 18, 24 and 36 h) groups (A and B). Toxicity of MCC950 in fibroblasts by CCK (C). Collagen-I and αSMA expression was measured by Western blot analyssay in cardiac fibroblast in the SHAM, Hypoxia and H + MCC950 (1, 10, 20, 40uM) (D and E). Representative and quantitative analysis of NLRP3, cleaved caspase-1, cleaved IL-1βand IL-18 levels demonstrate the corresponding differences in penumbral region after treatment with MCC950 (1,10,20 and 40uM) (F and G). MCC950 treatment reduced NLRP3, cleaved caspase-1, cleaved IL-1β and IL-18 expression (H). Data are depicted as Mean ± SEM. Differs significantly (one-way ANOVA with a post-hoc Holm-Sidak test, $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$, NS, not significant, compared with the Sham group; $^{*}P < 0.05$, $^{***}P < 0.001$, compared with the MI + PBS group).

Acknowledgements

None.

Funding

This research was funded by the Jiangxi Provincial Natural Science Foundation Project (20171BBG70067, 20181074, 2016YNQN12036). This is work was supported by National Natural Science Foundation of China (#81160019 , #81360031) and Nanchang Univertisity Innovation Foundation (CX2018188).

Conflict of interest statement

The authors declare that there is no conflict of interest.

References

[1] A. Abbate, F.N. Salloum, E. Vecile, A. Das, N.N. Hoke, S. Straino, et al., Anakinra, a recombinant human Interleukin-1 receptor antagonist, inhibits apoptosis in experimental acute myocardial infarction, Circulation 117 (20) (2008) 2670–2683.
[2] Z.J. Eapen, W.H.W. Tang, G.M. Felker, A.F. Hernandez, K.W. Mahaffey, A.M. Lincoff, et al., Defining heart failure end points in ST-segment elevation myocardial infarction trials: integrating past experiences to chart a path forward, Circulation 5 (4) (2012) 594–600.
[3] R.G. Gourdie, S. Dimmeler, P. Kohl, Novel therapeutic strategies targeting fibroblasts and fibrosis in heart disease, Nat. Rev. Drug Discov. 15 (9) (2016) 620–638.
[4] S. Toldo, A. Abbate, The NLRP3 inflammasome in acute myocardial infarction, Nat. Rev. Cardiol. 15 (4) (2018) 203–214.
[5] E. Mezzaroma, S. Toldo, D. Farkas, I.M. Seropian, B.W. Van Tassell, F.N. Salloum, et al., The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse, Proc. Natl. Acad. Sci. 108 (49) (2011) 19725–19730.
[6] M. Takahashi, NLRP3 inflammasome as a novel player in myocardial infarction, Int. Heart J. 55 (2) (2014) 101–105.
[7] E. Latz, T.S. Xiao, A. Stutz, Activation and regulation of the inflammasomes, Nat. Rev. Immunol. 13 (6) (2013) 397–411.
[9] M. Kawaguchi, M. Takahashi, T. Hata, Y. Kashima, F. Usui, H. Morimoto, et al., Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury, Circulation 123 (6) (2011) 594–604.
[10] S. Toldo, E. Mezzaroma, A.G. Mauro, F. Salloum, B.W. Van Tassell, A. Abbate, The inflammasome in myocardial injury and cardiac remodeling, Antioxid. Redox Signal. 22 (13) (2015) 1146–1161.
[11] M.T. Heneka, M.P. Kummer, E. Latz, Innate immune activation in neurodegenerative disease, Nat. Rev. Immunol. 14 (7) (2014) 463–477.
[12] M. Lamkanfi, V.M. Dixit, Inflammasomes and their roles in health and disease, Annu. Rev. Cell Dev. Biol. 28 (2012) 137–161.
[13] N.A. Bracey, P.L. Beck, D.A. Muruve, S.A. Hirota, J. Guo, H. Jabagi, et al., The Nlrp3 inflammasome promotes myocardial dysfunction in structural cardiomyopathy through interleukin-1β, Exp. Physiol. 98 (2) (2013) 462–472.
[15] W. Zhou, C. Chen, Z. Chen, L. Liu, J. Jiang, Z. Wu, et al., NLRP3: a novel mediator in cardiovascular disease, J Immunol Res 2018 (2018) 5702103.
[16] R.C. Coll, A.A.B. Robertson, J.J. Chae, S.C. Higgins, R. Muñoz-Planillo, M.C. Inserra, et al., A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases, Nat. Med. 21 (3) (2015) 248–255.
[17] C.E. Sutton, S.J. Lalor, C.M. Sweeney, C.F. Brereton, E.C. Lavelle, K.H. Mills, Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity, Immunity 31 (2) (2009) 331–341.
[18] B.Z. Shao, Z.Q. Xu, B.Z. Han, D.F. Su, C. Liu, NLRP3 inflammasome and its inhibitors: a review, Front. Pharmacol. 6 (2015) 262.
[19] G.P.J. van Hout, L. Bosch, G.H.J.M. Ellenbroek, J.J. de Haan, W.W. van Solinge, M.A. Cooper, et al., The selective NLRP3-inflammasome inhibitor MCC950 reduces infarct size and preserves cardiac function in a pig model of myocardial infarction, Eur. Heart J. 38 (11) (2017) 828–836.
[20] H. Mao, P. Su, W. Qiu, L. Huang, H. Yu, Y. Wang, The use of Masson's trichrome staining, second harmonic imaging and two-photon excited fluorescence of collagen in distinguishing intestinal tuberculosis from Crohn's disease, Color. Dis. 18 (12) (2016) 1172–1178.
[21] S.L. Carroll, R. Horowits, Myofibrillogenesis and formation of cell contacts mediate the localization of N-RAP in cultured chick cardiomyocytes, Cell Motil. Cytoskeleton 47 (1) (2000) 63–76.
[22] A. Dhume, S. Lu, R. Horowits, Targeted disruption of N-RAP gene function by RNA interference: a role for N-RAP in myofibril organization, Cell Motil. Cytoskeleton 63 (8) (2006) 493–511.
[23] A. Tzovaras, A. Kladi-Skandali, K. Michaelidou, G.C. Zografos, I. Missitzis, A. Ardavanis, et al., BCL2L12: a promising molecular prognostic biomarker in breast cancer, Clin. Biochem. 47 (18) (2014) 257–262.
[24] T. van der Heijden, E. Kritikou, W. Venema, J. van Duijn, P.J. van Santbrink, B. Slütter, et al., NLRP3 Inflammasome inhibition by MCC950 reduces atherosclerotic lesion development in apolipoprotein E–deficient mice—brief report, Arterioscler. Thromb. Vasc. Biol. 37 (8) (2017) 1457–1461.
[25] W. Gan, J. Ren, T. Li, S. Lv, C. Li, Z. Liu, et al., The SGK1 inhibitor EMD638683, prevents angiotensin II-induced cardiac inflammation and fibrosis by blocking NLRP3 inflammasome activation, Biochim. Biophys. Acta 1864 (1) (2018) 1–10.
[26] Z.J. Eapen, W.H. Tang, G.M. Felker, A.F. Hernandez, K.W. Mahaffey, A.M. Lincoff, et al., Defining heart failure end points in ST-segment elevation myocardial infarction trials: integrating past experiences to chart a path forward, Circ. Cardiovasc. Qual. Outcomes 5 (4) (2012) 594–600.
[27] N.G. Frangogiannis, Regulation of the inflammatory response in cardiac repair, Circ. Res. 110 (1) (2012) 159–173.
[28] O. Gross, C.J. Thomas, G. Guarda, J. Tschopp, The inflammasome: an integrated view, Immunol. Rev. 243 (1) (2011) 136–151.
[29] H.K. Min, A. Kapoor, M. Fuchs, F. Mirshahi, H. Zhou, J. Maher, et al., Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease, Cell Metab. 15 (5) (2012) 665–674.
[30] Z.Z. Ma, L.G. Lu, Cholesterol metabolism and non-alcoholic steatohepatitis, Zhonghua Gan Zang Bing Za Zhi 24 (8) (2016) 623–627.
[31] B.J. Pomerantz, L.L. Reznikov, A.H. Harken, C.A. Dinarello, Inhibition of caspase 1 reduces human myocardial ischemic dysfunction via inhibition of IL-18 and IL-1beta, Proc. Natl. Acad. Sci. U. S. A. 98 (5) (2001) 2871–2876.
[32] K. Venkatachalam, S.D. Prabhu, V.S. Reddy, W.H. Boylston, A.J. Valente, B. Chandrasekar, Neutralization of interleukin-18 ameliorates ischemia/reperfusion-induced myocardial injury, J. Biol. Chem. 284 (12) (2009) 7853–7865.
[33] L.C. O'Brien, E. Mezzaroma, B.W. Van Tassell, C. Marchetti, S. Carbone, A. Abbate, et al., Interleukin-18 as a therapeutic target in acute myocardial infarction and heart failure, Mol. Med. 20 (1) (2014) 221–229.
[34] T.C.T.M. van der Pouw Kraan, F.J.P. Bernink, C. Yildirim, P. Koolwijk, J.M. Baggen, L. Timmers, et al., Systemic toll-like receptor and interleukin-18 pathway activation in patients with acute ST elevation myocardial infarction, J. Mol. Cell. Cardiol. 67 (2014) 94–102.
[35] A. Kumar, V. Thota, L. Dee, J. Olson, E. Uretz, J.E. Parrillo, Tumor necrosis factor alpha and interleukin 1beta are responsible for in vitro myocardial cell depression induced by human septic shock serum, J. Exp. Med. 183 (3) (1996) 949–958.
[36] S. Toldo, E. Mezzaroma, L. O'Brien, C. Marchetti, I.M. Seropian, N.F. Voelkel, et al., Interleukin-18 mediates interleukin-1-induced cardiac dysfunction, Am. J. Physiol. Heart Circ. Physiol. 306 (7) (2014) H1025–H1031.
[37] E. Mezzaroma, S. Toldo, D. Farkas, I.M. Seropian, B.W. Van Tassell, F.N. Salloum, et al., The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse, Proc. Natl. Acad. Sci. U. S. A. 108 (49) (2011) 19725–19730.
[38] A. Saxena, W. Chen, Y. Su, V. Rai, O.U. Uche, N. Li, et al., IL-1 induces proinflammatory leukocyte infiltration and regulates fibroblast phenotype in the infarcted myocardium, J. Immunol. 191 (9) (2013) 4838–4848.
[39] M. Kawaguchi, M. Takahashi, T. Hata, Y. Kashima, F. Usui, H. Morimoto, et al., Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury, Circulation 123 (6) (2011) 594–604.
[40] S. Toldo, E. Mezzaroma, B.W. Van Tassell, D. Farkas, C. Marchetti, N.F. Voelkel, et al., Interleukin-1β blockade improves cardiac remodelling after myocardial infarction without interrupting the inflammasome in the mouse, Exp. Physiol. 98 (3) (2013) 734–745.