

BRD4 inhibition by JQ1 protects against LPS-Induced Cardiac Dysfunction by inhibiting SIRT1-dependent activation of NLRP3 inflammasomes

wenjun Li (✉ 1106273062@qq.com)

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

XiuFeng Shen

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

Shenglan Feng

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

Yue Liu

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

Huiying Zhao

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

Guohao Zhou

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

Ming Sang

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

Xiaodong Sun

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

Rong Jiao

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine

Fuyuan Liu

Xiangyang No 1 People's Hospital Affiliated to Hubei University of Medicine <https://orcid.org/0000-0001-9762-8168>

Research Article

Keywords: JQ1, BRD4, NLRP3, inflammation, Sirt1, cardiac dysfunction

Posted Date: February 28th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1328889/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

JQ1, a BRD4 protein inhibitor, first identified because of its therapeutic role in cancer, has gradually demonstrated a protective effect on the heart in recent years; however, it is unclear whether JQ1 also plays a role in LPS-induced cardiac dysfunction. This paper aims to investigate the effects of the BRD4 inhibitor JQ1 on LPS-induced cardiac dysfunction and its mechanism. In the experiments, we found that BRD4 was significantly upregulated in the hearts of LPS-treated mice. JQ1 treatment improved survival and cardiac function in LPS-treated mice and reduced cardiomyopathologic injury, inflammation, and oxidative injury. JQ1 treatment similarly reduced the release of lactate dehydrogenase and inflammatory factors in H9C2 cells treated with LPS. JQ1 significantly upregulated silent information regulator 1 (SIRT1) expression and suppressed the upregulation of NOD-like receptor protein 3 (NLRP3), cleaved caspase-1, and GSDMD in heart tissues induced by LPS. Meanwhile, we obtained the same results in H9C2 cells treated with LPS. The administration of the SIRT1 inhibitor (EX527) intervention partially blocked the JQ1-mediated downregulation of NLRP3, cleaved caspase-1, GSDMD in LPS-induced H9C2 cells. Therefore, we propose that JQ1 can improve LPS-induced cardiac dysfunction by inhibiting SIRT1-dependent activation of NLRP3 inflammasomes, which may be a promising strategy for treating sepsis cardiomyopathy.

1. Introduction

Sepsis is a complex multi-organ dysfunction syndrome. Cardiac contraction and diastolic dysfunction are often seen in patients with severe sepsis [18]. It should be noted that cardiovascular disease remains a significant cause of morbidity and mortality worldwide [6]. Lipopolysaccharide (LPS), a significant component of the outer membrane of Gram-negative bacteria that can trigger pathologies such as systemic inflammatory response syndrome (SIRS) and septic shock, has been widely used in the establishment of models of cardiac dysfunction in sepsis [20]. Therefore, it is crucial to explore the mechanisms of LPS-induced cardiac dysfunction.

NOD-like receptor protein 3 (NLRP3) is an intracellular protein complex that regulates the body's inflammatory response [14]. At the onset of inflammation, The NLRP3 is rapidly triggered for activation, activated NLRP3 binds to apoptosis-associated speck-like protein (ASC), then recruits caspase 1 to assemble into an inflammasome complex prompting the release of proinflammatory cytokines IL-1 β and IL-18 and upregulating GSDMD, leading to pyroptosis [15, 17, 34]. Previous studies have shown that NLRP3 inflammasome activation in cardiac fibroblasts during sepsis-induced maturation and release the inflammatory factors such as IL-1 β , inhibiting activation of the NLRP3 inflammasome in myocardial fibroblasts, can alleviate LPS-induced myocardial dysfunction and improve survival in mice with peritonitis sepsis [40]. Similarly, carbon monoxide molecules can also alleviate myocardial dysfunction in sepsis mice by inhibiting activation of NLRP3 inflammasome [39]. Silent information regulator 1 (SIRT1) is a member of the Sirtuins family and is a conserved nicotinamide adenine dinucleotide (NAD $^{+}$) - dependent histone deacetylase. SIRT1 is primarily located in the mammalian nucleus and exerts its

functions through acetylation activity. Recent studies have shown that SIRT1 can regulate NLRP3 to inhibit the onset of inflammation and delay the inflammatory response[13, 16, 24].

Bromodomain-containing protein 4 (BRD4) belongs to the bromine and extra-terminal domain (BET) protein families. It binds to acetylated histones and transcription factors through its bromide domain to regulate various pathophysiological activities, including inflammation and cancer[25, 29]. JQ1 is one of the bromine domain inhibitors of BRD4 and has a therapeutic effect on various diseases[1, 7, 19]. In recent years, there has been increasing evidence that JQ1 plays a role in cardiovascular disease. In cardiovascular animal models, JQ1 can reduce myocardial injury caused by ligation of the left coronary artery[32], reduce pulmonary hypertension, and prevent diabetic cardiomyopathy induced by a high-fat diet[22]. Taken together, these data suggest that the BRD4 protein is involved in cardiovascular disease. Recently, studies have shown that JQ1 is an effective regulator of SIRT1. In animal models, JQ1 can regulate autophagy, apoptosis, oxidative stress, and other cellular responses by upregulating SIRT1[7, 11, 27, 37]. However, the role of SIRT1 in the protection of JQ1 against LPS-induced cardiac dysfunction is unclear. Therefore, this study investigates whether BRD4 protein inhibitor JQ1 regulates LPS-induced NLRP3 activation in mice by activating SIRT1, thus playing a protective role in against LPS-induced cardiac dysfunction.

2. Materials And Methods

2.1 Grouping and treatment of experimental animals

C57BL/6J male mice, aged 8-10 weeks(20-25 g), were purchased from the Institute of Experimental Animal Science, Hubei Medical University(Shiyan, China). Before the experiments, the animals were kept in a thermostatic environment with regular 12-hour light/dark cycles and provided with standard food and water. Forty-eight mice were randomly divided into 3 groups (6 mice in each group):(1) control group, (2) LPS group, (3) JQ1+ LPS group. Mice in LPS group were intraperitoneally injected with LPS 7.5mg/Kg(from Escherichia coli,serotype 0111: B4,Sigma,dissolved in 0.9% NaCl). Mice in the LPS+JQ1 group were given a protective treatment with 50mg/Kg JQ1(MedChem Express, HY-13030, and dissolved in 10% DMSO) intravenously 1 hour before LPS stimulation. The dose of JQ1 was based on a previous study[31]. Mice in the control group were injected with DMSO in the caudal vein. Six mice in each group were euthanized after 12 h of LPS stimulation. Serum and heart tissue were collected and stored at -20°Cfor subsequent experiments(Fig. 1). The survival rate of the remaining mice was recorded every 12 h until 72 h after LPS stimulation.

2.2 Echocardiography

Cardiac function was assessed by echocardiography after 12 hours of LPS stimulation. Mice were anaesthetized with sevoflurane and fixed in the supine position. Echocardiography was collected using a Mindray Resona7 imaging system equipped with a 20 MHz linear transducer. Left ventricular end-diastolic diameter (LVDD) and left ventricular end-systolic diameter (LVSD) of mice in each group were detected, and left ventricular ejection fraction (LVEF) and left ventricular short-axis shorting rate (LVFS)

were calculated according to the built-in program. The mean values of the three measurements were recorded.

2.3 Measurement of Creatine Kinase-MB(CK-MB), Lactate Dehydrogenase (LDH), IL-6, IL-18

Serum and cell supernatants were collected and CK-MB, LDH, IL-1 β and IL-18 levels in serum and cell supernatants according to instructions. The CK-MB and LDH kits were purchased from Nanjing Jiancheng Bioengineering Institute, China. The IL-1 β ELISA kit and the IL-18 ELISA kit were purchased from MULTISCIENCES (LIANKE) BIOTECH, CO., LTD.

2.4 Oxidation index determination

The activity of superoxide dismutase (SOD), the activity of glutathione peroxidase (GSH-Px), and the content of malonaldehyde (MDA) were determined using the corresponding kit (Nanjing Jianchen Bioengineering Institute, China). After various treatments of cardiomyocytes and cardiac tissue, the cells and tissue homogenates were collected and fragmented in 500 μ l PBS using an ultrasonic crush, followed by centrifugation at 1200 rpm at 4°C for 10 min. The supernatant was collected to determine SOD activity, GSH-Px activity, and MDA content using the corresponding kit according to the manufacturer's instructions.

2.5 Histological Staining

The rats were killed by neck removal, and the cardiac tissue was obtained. The cardiac tissue was fixed with 4% (φ) paraformaldehyde, and then paraffin sections were prepared. Then HE staining was performed (baking slices - xylene dewaxing hydration - hematoxylin staining - acid ethanol solution differentiation - ethanol dehydration and transparency - neutral gum sealing slices). Finally, the changes in cardiac tissue were observed under a microscope. Three hearts were analyzed in each group.

2.6 Tissue immunofluorescence staining

Heart tissue fixed in 10% formalin was dehydrated, transparent, waxed in, embedded, fixed on a slicer, and cut into 5 μ m thick paraffin sections. Overnight with anti-CD45 antibody (1:1000, Proteintech) and then labeled with Goat anti-rabbit IgG H&L (1:2000, Abcam) secondary antibody for 30 min and Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) for 10 min. Immunocellular infiltration within the myocardium was visualized by confocal microscopy.

2.7 Cell culture and treatment

H9C2 myoblast cells were purchased from ProCell Life Science & Technology Co., Ltd. H9C2 cells were cultured in a DMEM medium (containing 10% serum, 100 ug/L penicillin, 100 ug/L streptomycin) in an incubator at 37°C and 5% CO₂ saturated humidity. The cells were divided into 4 groups: control group, LPS group, LPS+ JQ1 group, and JQ1+LPS + EX527 group. Cells were stimulated by LPS (10 μ g/mL) for

12h, and JQ1(0.5μm) and/or EX527(1 mM, Selleck Chemicals, Item No S1541) were added 1h before LPS for pre-protection.

2.8 CCK8 assay

H9C2 cells in the logarithmic growth stage and good growth state were inoculated into 96-well plates at a density of 3×10⁴/ mL.JQ1 with final concentrations of 0.25, 0.5, 1, 2, and 4μM were added into the cells. H9C2 cells supplemented with DMEM medium were set as the negative control group, and DMEM medium was set as the blank group. Each group has 3 duplicate holes. After 48h of culture, the absorbance (OD) of each well was measured at 450 nm using an automatic enzyme plate analyzer. The inhibitory rate of cell growth was calculated, and the inhibitory rate (%)=[1 –(OD value of the experimental group – OD value of the blank group)/(OD value of the control group – OD value of the blank group)]×100%.

2.9 Fluorescent staining of reactive oxygen species (ROS)

H9C2 cells were seeded in 12-well plates, and the media was discarded after 12 h of drug intervention.DCFH-DA probes were diluted with serum-free medium with 4 ',6-diamine-2-benzene-index (DAPI) blue in 1:1500, and incubated in the incubator for 30 min. The medium was discarded, and the cells were washed twice with a serum-free medium. The staining level of cells was observed under fluorescence microscope, and the fluorescence luminosity value was detected.

2.10 Western Blotting

The expression levels of BRD4, NLRP3, and SIRT1 in heart tissue and H9C2 cells were determined by Western Blotting. The protein samples were electrophoretic with 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes at 200 mA constant current. Then, after sealing with 5% skim milk powder at room temperature for 1 h, the anti-BRD4(1:1000;Abcam,ab128874),anti-SIRT1(1:1000;Abcam,ab189494), anti-NLRP3(1:1000;Cell Signaling Technology,15101S), anti-Caspase-1(1:1000; Cell Signaling Technology,3866T), anti-Cleaved caspase-1(1:1000;Cell Signaling Technology,4199T),anti-GSDMD(1:1000;Abclonal, A18281), and anti-GAPDH(1:1000,Absin,abs132004)were incubated at 4°Covernight.The membrane was washed three times and then incubated with secondary antibody at room temperature for 1 h. The protein bands were revealed by ECL (Beyotime Institute of Biotechnology, China) chemiluminescence solution. The Image J Image analysis system (NIH, USA) was used to quantify protein expression.GAPDH is used as an internal reference for Western Blotting.

2.11 Real-time PCR analysis

Total RNA was extracted from mouse myocardial tissue and H9C2 cells with Trizol reagent. cDNA was synthesized from total RNA using a reverse transcription kit. Amplification and quantitative RT-PCR analysis were performed using 7300 Real-Time PCR System (A p p l i e d B i o s y s t e m s) on a SYBR Green. The primer sequences used are as follows: 5'- CCATGGACATGAGCACAACTC- 3'(forward primer)

and 5'- TGGAGAACATCAATCGGACA- 3' (reverse primer) for the mouse BRD4 gene; 5'- CCGTGGCAAACCTGGTACTTT- 3'(forward primer) and 5'- GACGCCAACATAGACCACCT- 3' (reverse primer) for the mouse SIRT1 gene; 5'- GACA TGCCGCCTGGAGAAC - 3' (forward primer) and 5'- AGCCCAGGA TGCCCTTAGT- 3' (reverse primer) for GAPDH (used as an internal reference control). The results were quantified using the 2- $\Delta\Delta CT$ method.

2.12 Statistical Analyses

All data in our study were expressed as mean \pm SEM and statistically analyzed using GraphPad Prism software version 8.0. The comparison between the two groups was performed using the Two-tailed Student's t-test. Others used one-way analysis of variance (One-way ANOVA). P < 0.05 was considered statistically significant.

3. Result

3.1 Cardiac BRD4 expression is upregulated in LPS - treated mice

To determine the relationship between BRD4 and LPS-induced cardiac dysfunction, mice were intraperitoneally injected with LPS (7.5 mg/kg) for 12 hours. Then we took mice cardiac tissue; the levels of BRD4 were detected by Rt-PCR and Western Blotting. Before euthanasia, mice injected with LPS developed typical signs of septic shock, such as decreased motor activity, clammy skin, anorexia, and lethargy. The results showed that the mRNA (Fig. 2a) and protein expression (Fig. 2b, c) of BRD4 were significantly increased in the hearts of LPS-stimulated mice compared with the control group. These findings suggest that BRD4 protein may play a role in LPS-induced cardiac dysfunction.

3.2 BRD4 inhibitor JQ1 can improve the survival rate and cardiac dysfunction in LPS - treated mice

To test the effect of JQ1 on LPS-induced cardiac dysfunction in mice, we administered JQ1 through intravenous tail injection for pre protection for 1 hour, followed by intraperitoneal injection of LPS 7.5mg/Kg to induce myocardial injury. As shown in Figure 3A, the 72-h survival of LPS mice decreased to 41.6% compared to the control group. In contrast, treatment with 50mg /kg JQ1 significantly improved the survival rate to 75%. Echocardiography showed that LPS significantly reduced EF, FS, while treatment with JQ1 reversed these alterations (Fig. 3b, c, d).In addition, H&E staining of cardiac tissue revealed disrupted myocardial cell arrangement in the LPS group and increased cardiomyocytes compared to controls; in contrast, JQ1 treatment significantly alleviated these pathological abnormalities (Fig. 3b).LPS induced myocardial injury, as evidenced by significantly increased levels of LDH and CK-MB as compared to controls, and those of which were reduced by treatment with JQ1 (Fig. 3e, f). All these above data illustrate the protective effect of JQ1 on LPS-induced myocardial injury.

3.3 BRD4 inhibitor JQ1 reduces inflammation and oxidative damage in the heart tissue of LPS-treated mice

To assess the inflammatory response in mice, we performed immunofluorescence staining of infiltrating immune cells in myocardial tissue with a CD45 antibody and examined IL-1 β , IL-18 levels in serum. The results showed that in myocardial tissue, the number of CD45-positive infiltrating immune cells in myocardium and serum IL-1 β , IL-18 levels were significantly upregulated by LPS, and these effects were reversed by JQ1 (Fig. 4a, b, c). In addition, in assessing oxidative damage levels in heart tissue, we found that LPS treated mice had increased MDA content and significantly decreased SOD activity and GSH-PX activity, these alterations were similarly reversed by JQ1 treatment (Fig. 4d, e, f). These results demonstrate that JQ1 reduces inflammation and oxidative stress response in heart tissue of LPS treated mice.

3.4 BRD4 inhibitor JQ1 reduces LDH release, inflammation, and oxidative damage in LPS-treated H9C2 cells

To investigate the protective effect of JQ1 on LPS-treated H9C2 cells, cell damage indices, inflammatory factors, and oxidative damage indices were measured. In the CCK8 experiment, we found that JQ1 had no effect on cell viability at 0.25 and 0.5 μ M but decreased cell viability at 1 μ M (Fig. 5a). When the LDH level in the cell culture supernatant was measured, it was found that the LDH level decreased significantly when the JQ1 dose was 0.5 μ M (Fig. 5b). Therefore, 0.5 μ M JQ1 was selected as the optimal concentration for subsequent in vitro experiments. In addition, LPS treatment increased the release of IL-1 β and IL-18 in H9C2 cells, whereas JQ1 treatment significantly reversed these LPS-induced effects (Fig. 5c, d). In the evaluation of oxidative damage indexes, compared with the control group, ROS fluorescence was significantly enhanced after LPS induction, and SOD activity and GSH-Px activity were decreased. At the same time, JQ1 reversed these changes (Fig. 5e, f, g). These results indicate that JQ1 has a protective effect on LPS-treated H9C2 cells.

3.5 JQ1 upregulates SIRT1 expression and inhibits NLRP3 activation in vivo and in vitro

To explore the protective mechanism of JQ1 against LPS-induced myocardial injury, we detected the mRNA and protein expression levels of SIRT1, and it was found that LPS significantly reduced the mRNA and protein levels of SIRT1 in cardiac tissues, while JQ1 reversed these changes (Fig. 6a,b). At the same time, we detected the protein expression levels of NLRP3, Cleaved caspase-1, and GSDMD and found that LPS significantly upregulated the protein expression levels of NLRP3; Cleaved caspase-1, and GSDMD in cardiac tissues. However, JQ1 significantly inhibited LPS-induced up-regulation of NLRP3, Cleaved caspase-1, and GSDMD (Fig. 6b, c). In addition, we obtained the same results in H9C2 cells in vitro. In LPS-treated H9c2 cells, SIRT1 mRNA and protein levels were down-regulated, while JQ1 treatment upregulated SIRT1 (Fig. 6d, e). In LPS-treated H9C2 cells, protein expression levels of NLRP3, Cleaved caspase-1, and GSDMD were increased, while JQ1 treatment significantly reduced these changes (Fig. 6e, f). These results suggest that JQ1 upregulates SIRT1 expression and inhibits NLRP3 inflammasome activation in vivo and in vitro.

3.6 SIRT1 inhibitor antagonized the inhibitory effect of JQ1 on LPS-induced inflammation of H9C2 cells

To further confirm whether JQ1 inhibits the release of inflammatory factors in LPS-treated mice and H9C2 cells through the SIRT1 pathway, SIRT1 inhibitor EX527 was added to the experiment. The results showed that, as described above, the release of IL-1 β and IL-18 in LPS-treated H9C2 cells was significantly increased compared with the control group. JQ1 treatment inhibited the release of IL-1 β and IL-18, whereas EX527 blocked the release of JQ1-mediated inflammatory cytokines (Fig. 7a, b). In addition, LPS-treated H9C2 cells were down-regulated in NLRP3, Cleaved caspase-1, and GSDMD protein expressions, while JQ1 treatment inhibited such changes. The addition of EX527 blocked the inhibitory effect of JQ1 on NLRP3 inflammasome activation (Fig. 7c-g). These results suggest that JQ1 may ameliorate LPS-induced cardiac inflammation through the SIRT1 pathway.

4. Discussion

In this study, we confirmed that NLRP3 inflammasome activation plays a vital role in the pathogenesis of LPS-induced cardiac dysfunction, which is consistent with previous studies[3, 20, 21, 23]. This study is the first to demonstrate a protective effect of THE BRD4 inhibitor JQ1 on LPS-induced cardiac dysfunction. This study found that JQ1 significantly reduces cellular inflammation by inhibiting NLRP3 inflammasome activation in vivo and in vitro. In addition, we found that JQ1 increased SIRT1 expression in LPS-treated mice myocardium, and the inhibition of SIRT1 by EX527 partially eliminated the inhibition of JQ1 on NLRP3 inflammasome activation. This suggests that JQ1 inhibits the activation of NLRP3 inflammasome in mice by upregulating SIRT1, thereby protecting against LPS-induced cardiac dysfunction.

BRD4 inhibitor JQ1 was first discovered because of its anti-tumor effect, and in recent years, its anti-inflammatory effect has also been gradually discovered. In animal models, JQ1 treatment alleviates various inflammation, such as gastritis, osteoarthritis, pancreatitis, microglitis, etc. [4, 5, 10, 26, 35, 38, 42]In cardiovascular disease-related inflammation, JQ1 can alleviate myocardial infarction-induced inflammation by inhibiting the activation of TLR4 signal and the inflammatory response of vascular endothelial cells by inhibiting the MAPK/NF- κ B signaling pathway[33, 36]. However, the role of JQ1 in LPS-induced cardiac dysfunction remains unclear. Therefore, we established a mice model of LPS-induced cardiac dysfunction and found that JQ1 could reverse cardiac dysfunction, restore the damage of cardiac tissue structure, reduce the inflammatory cell infiltration, reduce the release of serum myocardial enzymes (LDH, CK-MB) and inflammatory factors (IL-1 β , IL-18), and reduce oxidative damage, and improve the survival rate. In addition, we obtained the same results in LPS-treated H9C2 cells. This suggests that JQ1 has a protective effect on LPS-induced cardiac dysfunction.

In the early stages of sepsis, a moderate inflammatory response can ward off foreign pathogens and prevent further tissue damage. However, excessive inflammation can lead to organ damage and dysfunction[9]. NLRP3 inflammasome is thought to be involved in the inflammatory cascade amplification of sepsis and has a particular value in predicting the severity of sepsis patients[8, 41]. Previous studies have suggested that NLRP3 inflammasome plays a vital role in maintaining normal cardiac function[43].When NLRP3 is activated, it is involved in the inflammatory response of the

myocardium, secreting IL-1 β and IL-18 and recruiting inflammatory cell infiltration, leading to myocardial injury[11]. Our results showed that NLRP3, Cleaved caspase-1, and GSDMD protein levels were increased in vitro and in vivo models. However, JQ1 significantly reduced the levels of NLRP3, Cleaved caspase-1, and GSDMD, and inhibited the production of IL-1 β and IL-18. Therefore, these data suggest that JQ1 protects against cardiac injury by inhibiting NLRP3 activation and inflammatory cytokine release.

SIRT1, a member of the sirtuin family, has histone deacetylase activity and can participate in many biological processes such as cell growth and apoptosis[2]. In recent years, it has been found that the damage of the SIRT1 signaling pathway is one of the critical mechanisms mediating cardiac injury in sepsis, and activation of the SIRT1 signaling pathway is expected to become an essential strategy for the prevention and treatment of cardiac injury in sepsis[30]. Han et al. found that compared with wild-type mice, the mortality and myocardial injury of SIRT1 $^{-/-}$ mice were significantly increased after cecal ligation perforation[12]. A recent study showed that the inhibition of BRD4 upregulates SIRT1 and restores impaired autophagy flow in an experimental model of acute pancreatitis[28]. In the model of cardiac dysfunction induced by LPS in this experiment, we observed that JQ1 likewise increased SIRT1 levels in cardiac tissue and H9C2 cells. We further investigated whether SIRT1 is involved in the inhibitory effect of JQ1 on NLRP3 inflammasome activation in H9C2 cells. In the experiments, we found that JQ1 reversed the LPS-induced reduction in SIRT1 levels and inhibited the activation of the NLRP3 inflammasome. Inhibition of BET protein has not been reported to not only expose more acetylation sites for SIRT1 deacetylase activity but also upregulate SIRT1 levels, thereby enhancing SIRT1 deacetylation modification that in turn affect gene transcription induced during the inflammatory response. To further investigate the relationship between SIRT1 and NLRP3, we intervened using a specific SIRT1 inhibitor of EX527 and showed that EX527 partially abolished the inhibitory effect of JQ1 on NLRP3 inflammasome activation. This suggests that SIRT1 may mediate the anti-inflammatory activity of JQ1 in LPS-induced cardiomyopathy.

Declarations

AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. Investigation, funding acquisition, data collection and analysis were performed by Fuyuan Liu and Rong Jiao. The first draft of the manuscript was written by Wenjun Li and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

FUNDING INFORMATION

This investigation was supported by The Foundation of Health Commission of Hubei Province (WJ2021F064), Innovative Research Program of Xiangyang No.1 People's Hospital (XYY2021S04).

COMPLIANCE WITH ETHICAL STANDARDS

All procedures were approved by the Animal Studies Ethics Committee of Xiangyang No.1 People's Hospital(Xiangyang, China) and were conducted following the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health(NIH Publication, revised 2011).

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

References

1. 2020) Selective Inhibition of BET Protein Domains Has Functional Relevance. *Cancer Discov* 10: 637
Doi 10.1158/2159-8290.CD-RW2020-048
2. Alves-Fernandes DK, Jasiulionis MG (2019) The Role of SIRT1 on DNA Damage Response and Epigenetic Alterations in Cancer. *Int J Mol Sci* 20 Doi 10.3390/ijms20133153
3. Arioz BI, Tastan B, Tarakcioglu E, Tufekci KU, Olcum M, Ersoy N, Bagriyanik A, Genc K, Genc S (2019) Melatonin Attenuates LPS-Induced Acute Depressive-Like Behaviors and Microglial NLRP3 Inflammasome Activation Through the SIRT1/Nrf2 Pathway. *Front Immunol* 10: 1511 Doi 10.3389/fimmu.2019.01511
4. Baek M, Yoo E, Choi HI, An GY, Chai JC, Lee YS, Jung KH, Chai YG (2021) The BET inhibitor attenuates the inflammatory response and cell migration in human microglial HMC3 cell line. *Sci Rep-Uk* 11: 8828 Doi 10.1038/s41598-021-87828-1
5. Chen L, Zhong X, Cao W, Mao M, Li W, Yang H, Li M, Shi M, Zhang Y, Deng Yet al (2021) JQ1 as a BRD4 Inhibitor Blocks Inflammatory Pyroptosis-Related Acute Colon Injury Induced by LPS. *Front Immunol* 12: 609319 Doi 10.3389/fimmu.2021.609319
6. Chen Y, Freedman ND, Albert PS, Huxley RR, Shiels MS, Withrow DR, Spillane S, Powell-Wiley TM, Berrington De González A (2019) Association of Cardiovascular Disease With Premature Mortality in the United States. *Jama Cardiol* 4: 1230-1238 Doi 10.1001/jamacardio.2019.3891
7. Civenni G, Bosotti R, Timpanaro A, Vàzquez R, Merulla J, Pandit S, Rossi S, Albino D, Allegrini S, Mitra Aet al (2019) Epigenetic Control of Mitochondrial Fission Enables Self-Renewal of Stem-like Tumor Cells in Human Prostate Cancer. *Cell Metab* 30: 303-318 Doi 10.1016/j.cmet.2019.05.004
8. Danielski LG, Giustina AD, Bonfante S, Barichello T, Petronilho F (2020) The NLRP3 Inflammasome and Its Role in Sepsis Development. *Inflammation* 43: 24-31 Doi 10.1007/s10753-019-01124-9
9. Esquerdo KF, Sharma NK, Brunialti M, Baggio-Zappia GL, Assunção M, Azevedo L, Bafi AT, Salomao R (2017) Inflammasome gene profile is modulated in septic patients, with a greater magnitude in non-survivors. *Clin Exp Immunol* 189: 232-240 Doi 10.1111/cei.12971
10. Fukui T, Yik J, Doyran B, Davis J, Haudenschild AK, Adamopoulos IE, Han L, Haudenschild DR (2021) Bromodomain-containing-protein-4 and cyclin-dependent-kinase-9 inhibitors interact synergistically in vitro and combined treatment reduces post-traumatic osteoarthritis severity in mice. *Osteoarthr Cartilage* 29: 68-77 Doi 10.1016/j.joca.2020.07.012

11. Guo T, Jiang ZB, Tong ZY, Zhou Y, Chai XP, Xiao XZ (2020) Shikonin Ameliorates LPS-Induced Cardiac Dysfunction by SIRT1-Dependent Inhibition of NLRP3 Inflammasome. *Front Physiol* 11: 570441 Doi 10.3389/fphys.2020.570441
12. Han D, Li X, Li S, Su T, Fan L, Fan WS, Qiao HY, Chen JW, Fan MM, Li XJ et al (2017) Reduced silent information regulator 1 signaling exacerbates sepsis-induced myocardial injury and mitigates the protective effect of a liver X receptor agonist. *Free radical biology & medicine* 113: 291-303 Doi 10.1016/j.freeradbiomed.2017.10.005
13. Han Y, Sun W, Ren D, Zhang J, He Z, Fedorova J, Sun X, Han F, Li J (2020) SIRT1 agonism modulates cardiac NLRP3 inflammasome through pyruvate dehydrogenase during ischemia and reperfusion. *Redox Biol* 34: 101538 Doi 10.1016/j.redox.2020.101538
14. Huang Y, Xu W, Zhou R (2021) NLRP3 inflammasome activation and cell death. *Cell Mol Immunol* Doi 10.1038/s41423-021-00740-6
15. Hughes MM, O'Neill L (2018) Metabolic regulation of NLRP3. *Immunol Rev* 281: 88-98 Doi 10.1111/imr.12608
16. Jiao P, Li W, Shen L, Li Y, Yu L, Liu Z (2020) The protective effect of doxofylline against lipopolysaccharides (LPS)-induced activation of NLRP3 inflammasome is mediated by SIRT1 in human pulmonary bronchial epithelial cells. *Artificial cells, nanomedicine, and biotechnology* 48: 687-694 Doi 10.1080/21691401.2020.1730391
17. Jo EK, Kim JK, Shin DM, Sasakawa C (2016) Molecular mechanisms regulating NLRP3 inflammasome activation. *Cell Mol Immunol* 13: 148-159 Doi 10.1038/cmi.2015.95
18. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R (2015) Systemic inflammatory response syndrome criteria in defining severe sepsis. *The New England journal of medicine* 372: 1629-1638 Doi 10.1056/NEJMoa1415236
19. Korb E, Herre M, Zucker-Scharff I, Darnell RB, Allis CD (2015) BET protein Brd4 activates transcription in neurons and BET inhibitor Jq1 blocks memory in mice. *Nat Neurosci* 18: 1464-1473 Doi 10.1038/nn.4095
20. Li H, Zhou L, Zhi Z, Lv X, Wei Z, Zhang X, Tang W, Tong M (2020) Lipopolysaccharide upregulates miR-132/212 in Hirschsprung-associated enterocolitis, facilitating pyroptosis by activating NLRP3 inflammasome via targeting Sirtuin 1 (SIRT1). *Aging* 12: 18588-18602 Doi 10.18632/aging.103852
21. Liu X, Jin X, Yu D, Liu G (2019) Suppression of NLRP3 and NF-κB signaling pathways by α-Cyperone via activating SIRT1 contributes to attenuation of LPS-induced acute lung injury in mice. *Int Immunopharmacol* 76: 105886 Doi 10.1016/j.intimp.2019.105886
22. Mu J, Zhang D, Tian Y, Xie Z, Zou MH (2020) BRD4 inhibition by JQ1 prevents high-fat diet-induced diabetic cardiomyopathy by activating PINK1/Parkin-mediated mitophagy in vivo. *J Mol Cell Cardiol* 149: 1-14 Doi 10.1016/j.yjmcc.2020.09.003
23. Park S, Shin J, Bae J, Han D, Park SR, Shin J, Lee SK, Park HW (2020) SIRT1 Alleviates LPS-Induced IL-1β Production by Suppressing NLRP3 Inflammasome Activation and ROS Production in Trophoblasts. *Cells-Basel* 9 Doi 10.3390/cells9030728

24. Rahim I, Djerdjouri B, Sayed RK, Fernández-Ortiz M, Fernández-Gil B, Hidalgo-Gutiérrez A, López LC, Escames G, Reiter RJ, Acuña-Castroviejo D (2017) Melatonin administration to wild-type mice and nontreated NLRP3 mutant mice share similar inhibition of the inflammatory response during sepsis. *J Pineal Res* 63 Doi 10.1111/jpi.12410
25. Rahnamoun H, Lee J, Sun Z, Lu H, Ramsey KM, Komives EA, Lauberth SM (2018) RNAs interact with BRD4 to promote enhanced chromatin engagement and transcription activation. *Nat Struct Mol Biol* 25: 687-697 Doi 10.1038/s41594-018-0102-0
26. Segatto M, Szokoll R, Fittipaldi R, Bottino C, Nevi L, Mamchaoui K, Filippakopoulos P, Caretti G (2020) BETs inhibition attenuates oxidative stress and preserves muscle integrity in Duchenne muscular dystrophy. *Nat Commun* 11: 6108 Doi 10.1038/s41467-020-19839-x
27. Shen S, Li B, Dai J, Wu Z, He Y, Wen L, Wang X, Hu G (2020) BRD4 Inhibition Protects Against Acute Pancreatitis Through Restoring Impaired Autophagic Flux. *Front Pharmacol* 11: 618 Doi 10.3389/fphar.2020.00618
28. Shen S, Li B, Dai J, Wu Z, He Y, Wen L, Wang X, Hu G (2020) BRD4 Inhibition Protects Against Acute Pancreatitis Through Restoring Impaired Autophagic Flux. *Front Pharmacol* 11: 618 Doi 10.3389/fphar.2020.00618
29. Shi J, Vakoc CR (2014) The mechanisms behind the therapeutic activity of BET bromodomain inhibition. *Mol Cell* 54: 728-736 Doi 10.1016/j.molcel.2014.05.016
30. Smith LM, Yoza BK, Hoth JJ, McCall CE, Vachharajani V (2020) SIRT1 Mediates Septic Cardiomyopathy in a Murine Model of Polymicrobial Sepsis. *Shock (Augusta, Ga.)* 54: 96-101 Doi 10.1097/SHK.0000000000001429
31. Song S, Liu L, Yu Y, Zhang R, Li Y, Cao W, Xiao Y, Fang G, Li Z, Wang X et al (2019) Inhibition of BRD4 attenuates transverse aortic constriction- and TGF-β-induced endothelial-mesenchymal transition and cardiac fibrosis. *J Mol Cell Cardiol* 127: 83-96 Doi 10.1016/j.yjmcc.2018.12.002
32. Sun Y, Huang J, Song K (2015) BET protein inhibition mitigates acute myocardial infarction damage in rats via the TLR4/TRAFF/NF-κB pathway. *Exp Ther Med* 10: 2319-2324 Doi 10.3892/etm.2015.2789
33. Sun Y, Xie Y, Du L, Sun J, Liu Z (2018) Inhibition of BRD4 attenuates cardiomyocyte apoptosis via NF-κB pathway in a rat model of myocardial infarction. *Cardiovasc Ther* 36 Doi 10.1111/1755-5922.12320
34. Swanson KV, Deng M, Ting JP (2019) The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nature reviews. Immunology* 19: 477-489 Doi 10.1038/s41577-019-0165-0
35. Tan YF, Wang M, Chen ZY, Wang L, Liu XH (2020) Inhibition of BRD4 prevents proliferation and epithelial-mesenchymal transition in renal cell carcinoma via NLRP3 inflammasome-induced pyroptosis. *Cell Death Dis* 11: 239 Doi 10.1038/s41419-020-2431-2
36. Wang Q, Ozer HG, Wang B, Zhang M, Urabe G, Huang Y, Kent KC, Guo LW (2021) A hierarchical and collaborative BRD4/CEBPD partnership governs vascular smooth muscle cell inflammation.

37. Wen X, Klionsky DJ (2017) BRD4 is a newly characterized transcriptional regulator that represses autophagy and lysosomal function. *Autophagy* 13: 1801-1803 Doi 10.1080/15548627.2017.1364334
38. Zhang K, Xu Y (2020) Suppressing BRD4 exhibits protective effects against vincristine-induced peripheral neuropathy by alleviating inflammation and oxidative stress. *Biochem Bioph Res Co* 532: 271-279 Doi 10.1016/j.bbrc.2020.06.142
39. Zhang W, Tao A, Lan T, Cepinskas G, Kao R, Martin CM, Rui T (2017) Carbon monoxide releasing molecule-3 improves myocardial function in mice with sepsis by inhibiting NLRP3 inflammasome activation in cardiac fibroblasts. *Basic Res Cardiol* 112: 16 Doi 10.1007/s00395-017-0603-8
40. Zhang W, Xu X, Kao R, Mele T, Kviety P, Martin CM, Rui T (2014) Cardiac fibroblasts contribute to myocardial dysfunction in mice with sepsis: the role of NLRP3 inflammasome activation. *Plos One* 9: e107639 Doi 10.1371/journal.pone.0107639
41. Zhao S, Chen F, Yin Q, Wang D, Han W, Zhang Y (2020) Reactive Oxygen Species Interact With NLRP3 Inflammasomes and Are Involved in the Inflammation of Sepsis: From Mechanism to Treatment of Progression. *Front Physiol* 11: 571810 Doi 10.3389/fphys.2020.571810
42. Zhu F, Xiong F, He J, Liu K, You Y, Xu Q, Miao J, Du Y, Zhang L, Ren H et al (2020) Brd4 inhibition ameliorates Pyocyanin-mediated macrophage dysfunction via transcriptional repression of reactive oxygen and nitrogen free radical pathways. *Cell Death Dis* 11: 459 Doi 10.1038/s41419-020-2672-0
43. Zuurbier CJ (2019) NLRP3 Inflammasome in Cardioprotective Signaling. *J Cardiovasc Pharm* 74: 271-275 Doi 10.1097/FJC.0000000000000696

Figures

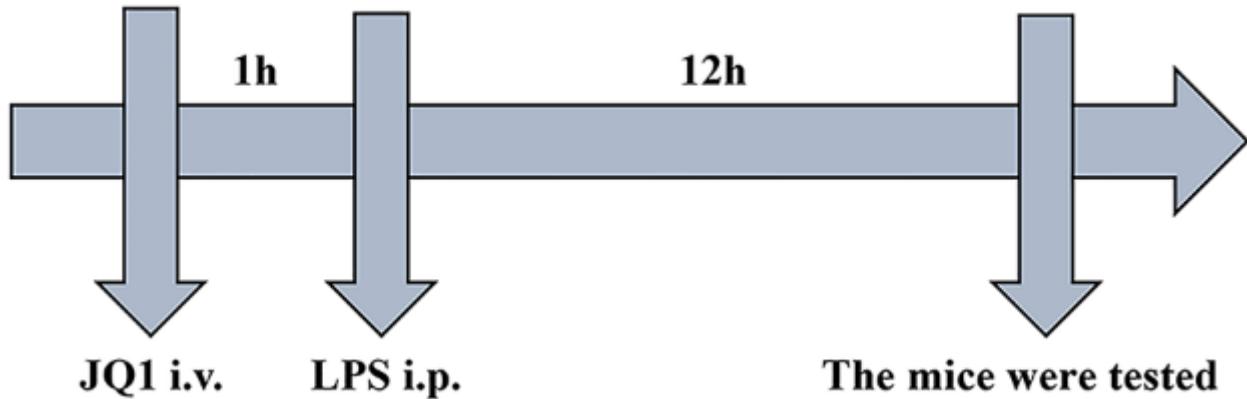


Figure 1

Treatment of animals in this study.

Figure 2

BRD4 expression was upregulated in the heart in LPS-induced cardiac dysfunction. (a) BRD4 mRNA levels of heart tissue in mice; (b) BRD4 expression of mice by Western-blot assay. (c) The ratio of BRD4 to 3-aldehyde glycerophosphate dehydrogenase (GAPDH) expression. All data are indicated as the mean±standard deviation (n=6). ***P<0.001, **P<0.01, *P<0.05.

Figure 3

JQ1 improved survival and improved cardiac dysfunction in LPS-treated mice. (a) Effect of JQ1 on survival of mice after LPS treatment (n=12 / group). (b) Effects of JQ1 on cardiac function and histomorphology in mice after LPS treatment. Echocardiography determined the cardiac functional parameters EF (c) and FS (d). Serum levels of CK-MB (e) and LDH (f) were determined in each group. All data are indicated as the mean±standard deviation (n=6). ***P<0.001, **P<0.01, *P<0.05.

Figure 4

JQ1 reduces inflammation and oxidative damage in the heart tissue of LPS-treated mice. (a) The CD45 was detected by immunofluorescence; Serum levels of IL-1 β (b) and IL-18 (c) were measured using the ELISA kit; Mice heart tissue GSH-PX viability (d), SOD viability (e), and MDA content (f). All data are indicated as the mean±standard deviation (n=6). ***P<0.001, **P<0.01, *P<0.05.

Figure 5

JQ1 reduces H9C2 LDH release, inflammation, and oxidative damage from LPS treatment. (a) Changes in cell viability after 12 h of different concentrations of JQ1 on H9C2 cells by CCK8 assay; (b) The release of LDH in the cell culture supernatants; The levels of IL-1 β (c) and IL-18 (d) of the cell culture supernatants were determined by the ELISA kit; GSH-PX viability (e) and SOD viability (f) were detected in H9C2 cells; (g) The ROS of the cells was detected by the fluorescence assay. All data are indicated as the mean±standard deviation (n=6). ***P<0.001, **P<0.01, *P<0.05.

Figure 6

JQ1 upregulates SIRT1 expression and inhibits NLRP3 activation in vivo and in vitro.(a, d) SIRT1 mRNA levels in mice heart tissue and H9C2 cells;(b, e) Expression of SIRT1, NLRP3, ProCasp-1, Casp-1p20, and GSDMD in heart tissue and H9C2 cells were measured by Western Blotting. (c, f) The ratio of SIRT1, NLRP3, ProCasp-1, Casp-1p20, and GSDMD to glyceraldehyde acid 3-phosphate dehydrogenase (GAPDH) expression in cardiac tissue and H9C2 cells.All data are indicated as the mean ± standard deviation (n=6).***P<0.001**P<0.01*P<0.05.

Figure 7

SIRT1 inhibitors can antagonize the inhibitory effect of JQ1 on LPS-induced H9C2 cell inflammation. (a, b) IL-1 β and IL-18 levels were determined in the supernatants of H9C2 cells treated with LPS by the ELISA assay;(c) SIRT1, NLRP3, ProCasp-1, Casp-1p20, and GSDMD expression was measured in H9C2 cells by the Western blotting assay. (d) The expression ratio of NLRP3 to GAPDH levels. (e)The expression ratio of ProCasp-1 to GAPDH levels;(f) The expression ratio of Casp-1p20 to GAPDH levels;(g) The expression ratio of GSDMD to GAPDH levels. All data are expressed as the mean ± standard deviation.***P<0.001**P<0.01*P<0.05.